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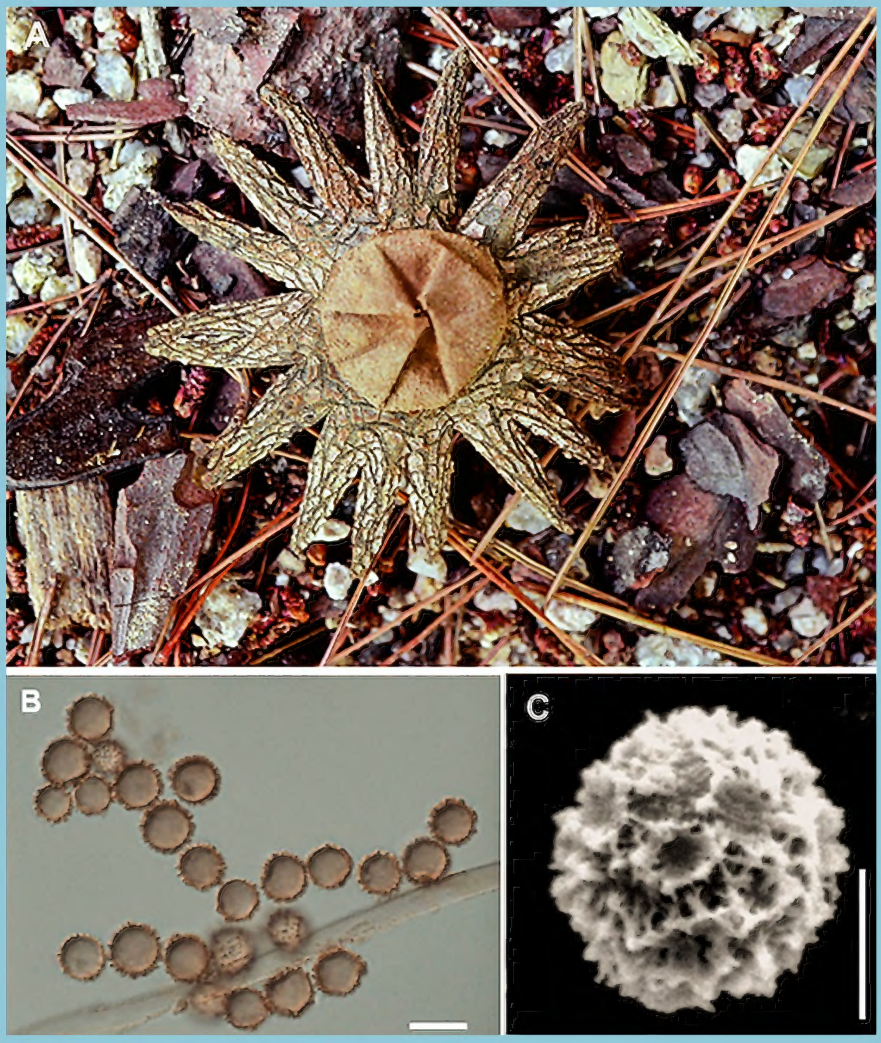

Astraeus ryoocheoninii sp. nov.
(Ryoo & al.— FIG. 2, p. 69)

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Mycotaxon *for* October–December 2016, (i–xii + 735–1000)
was issued on January 30, 2017

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Reviewers — volume one hundred thirty-two (1)

The Editors express their appreciation to the following individuals who have, prior to acceptance for publication, reviewed one or more of the papers prepared for this quarter.

Najam-ul-Sehar Afshan
Teuvo Ahti
M. Catherine Aime
Gerald L. Benny
Janusz Błaszkowski
Tiara S. Cabral
Rafael F. Castañeda-Ruíz
Pablo Pérez Daniëls
Cvetomir M. Denchev
José Carmine Dianese
Zai-Wei Ge
Nils Hallenberg
María L. Hernández Caffot
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Sidney Luiz Stürmer
Reinaldo Vargas Castillo
Josef Vlasák
Felipe Wartchow
Andrew W. Wilson
Li-Wei Zhou

From the Editor-in-Chief

Richard P. Korf in Full Color—Those wishing a full color printed version of our memoriam to Mycotaxon's co-founder may order a special copy of "Richard P. Korf (1925–2016): A Celebration" for $8 on the Mycotaxon website.

Surviving Co-founder Grégoire Hennebert recently corrected the 1973 date under the photo of the two founders in Louvain, noting that Dick first visited him in Belgium in 1972 on his Fulbright Fellowship. During Dick's stay at the Laboratory of Systematic Mycology at Louvain University where he worked with Grégoire on *Botrytis* and conidial states of *Sclerotiniaceae* (see Hennebert, '*Botrytis* and *Botrytis*-like genera', Persoonia 1973), Grégoire expressed a desire to launch a new mycological journal (named 'Biourgia' for the Belgian *Penicillium* specialist, Prof. Biourge) "because I had lost priority of one new species due to slow publication in a traditional scientific journal."

The two colleagues later discussed setting up a new journal during Grégoire's Sep. 23–30 1973 visit with Dick at Cornell in Ithaca and his Nov. 5–Dec. 14 1973 stint as a Visiting Professor in Canada and the United States. Feeling that the editorial work might be too time-consuming for Grégoire (who had "no hope for assistance in Louvain"), Dick suggested serving as Co-editor. He had previously published a few papers in Phytologia, an offset botanical publication and felt that editing ready-to-print manuscripts would be a time-saving solution. Dick proposed a more international title, Mycotaxon, for their journal and that he manage the edition from Ithaca, with Grégoire agreeing to control "the book review job."

At the time Grégoire still had no electric typewriter in Louvain, and so manually prepared his paper (with fully justified lines) on *Lomentospora prolifica,* a new hyphomycete, for Mycotaxon's first issue (1974, Mycotaxon 1(1): 45–50), which was, according to Grégoire, "a real hard job, a problem soon solved by the computer." [Younger readers may not realize how much time and paper it took to line up each right-hand margin, involving multiple attempts at spacing words with the "/" key at the margin before inserting interior spaces in the old Courier font where every letter and space had the same width.] Many thanks to Grégoire for these 'ancient' historical notes!

Break with tradition—Since its inception, Mycotaxon has presented its 'nomenclatural novelties' at the close of each volume (1974–2014 p.p.) or issue (2014 ff.). Our 'nom novs' section (which lists new combinations as well as names new to science and expanded to incorporate typifications in 2012) has until now appeared online as a separate free-access PDF. However, it makes more economic sense to absorb the novelties within the separate cover section (also free access). To aid those who will—no doubt—continue to search the end of each issue for the 'new' nomenclature, we now entitle our cover sections with the issue number followed by "Table of contents, nomenclatural updates, peers & editorials." This issue's newly proposed names (with authorities, nomenclatural identification numbers, and page where published) are listed on p. vii of this free access 132(1) cover section.

New requirements for range extension papers—Your editors have fretted for some time over problems presented by papers reporting on species outside of

formerly published distribution ranges. One problem has been the presentation of capsule 'field-guide type' descriptions that do not describe the specimens in sufficient detail to demonstrate that they represent the species to which they are referred. In keeping with our taxonomic focus, we alert authors to the necessity of devoting as much care to range extension descriptions as they do for a new species and ask them to point out differences that might indicate the existence of a cryptic taxon warranting research by an expert.

To that end, we have developed the following requirements for authors intending to publish a previously described taxon in Mycotaxon.

An acceptable taxonomic contribution must include an original description and illustration(s) of the newly collected specimens. Additionally, for each taxon range extension, authors must include:

[1] a taxonomic heading including the authorship, bibliographic reference, and the figure references;

[2] an original technical description based entirely on the newly collected specimens (no information from previously published descriptions should be included in this first description);

[3] a standard Specimens examined section, listing the collection and vouchering data for the newly collected specimens; and

[4] a brief discussion of similarities/differences between the newly collected specimens and previously published descriptions and data from the same species.

2017 guides & forms—Prospective authors should obtain the 2017 guide, sample manuscript, blank template and peer reviewer and submissions forms from Mycotaxon's Author Download page on <www.mycotaxon.com>. Note: there are two different sets of forms: one for papers to be published in the journal (requiring two expert reviewers) and a second set for annotated species lists (mycobiotas) to be posted on our Regional Checklist Page (requiring three expert reviewers).

Mycotaxon 132(1) contains 26 research papers by 94 authors (representing 23 countries) and revised by 49 expert reviewers.

Within its pages are 10 species new to science [representing *Astraeus* from Korea; *Chloridium* and *Circinella* from China; *Cyanodermella* from an Austrian cultivar; *Lembosia* and *Mycelephas* from Brazil; *Leucoagaricus* from Italy; *Phlebia* from India; and *Scutellospora* from Venezuela] and new combinations in *Caloplaca, Colletotrichum,* and *Puccinia.*

In addition to range extensions [lichens in Bolivia, Poland, and Turkey; polypores in China; powdery mildew in Korea; rust in Pakistan; puffballs in Thailand] and a new host for *Puccinia menthae* in Turkey, we also offer the complete life cycle for a rust in Ecuador, excellent coverage of five penzigioid *Xylaria* species in Vera Cruz, and molecular confirmation of *Geastrum reinkingii* as an independent species.

Warm regards,
Lorelei L. Norvell (*Editor-in-Chief*)
21 April 2017

2017 Mycotaxon submission procedure

Prospective Mycotaxon authors should download the Mycotaxon 2017 guide, review & submission forms, and Mycotaxon sample manuscript by clicking the 'file download page' link on our Instructions to Authors page before preparing their manuscript. This page summarizes our '4-step' submission process.

1—Peer review: Authors first contact two (for journal papers) or three (for annotated species 'weblists') peer reviewers before sending them formatted text & illustration files and the appropriate 2017 Mycotaxon journal or weblist reviewer comment form. Experts return revisions & comments to both the *Editor-in-Chief* <editor@mycotaxon.com> and authors. The authors correct their files before submitting their manuscript to the *Nomenclature Editor*.

2—Nomenclatural review: Email all **error-free** text and illustration files to the *Nomenclature Editor* <PennycookS@LandcareResearch.co.nz>. Place **first author surname** + **genus** + 'Mycotaxon' on the subject line; list title & coauthors and peer reviewer names & Email addresses in the message. The Nomenclature Editor will (i) reply immediately with the accession number and (2) return his notes with suggested revisions to the author(s) and *Editor-in-Chief* after a few weeks.

3—Final submission: Authors should consult experts and thoroughly revise and proof-read manuscripts to prepare error-free text and image files ready for immediate publication. **Label** the final submission email to the *Editor-in-Chief* <editor@mycotaxon.com> and **all** files & forms with **accession number;** attach the **(i)** 2017 submission form, **(ii)** labeled text files, **(iii)** labeled jpg files, and [required] **(iv)** FN, IF, or MB identification verification for each new name. The *Editor-in-Chief* usually contacts all coauthors and expert reviewers within two weeks of final submission, but please wait at least 14 days before sending a follow-up query (without attachments).

4—Final editorial review: Files with errors will be rejected or returned for revision. The *Editor-in-Chief* conducts a final grammatical and scientific review and returns her editorial revisions to all expert reviewers and coauthors for final author approval. Author-approved files are placed in the publication queue.

The PDF proof and bibliographic & nomenclatural index entries will be sent to all coauthors for final inspection. After PDF processing, the *Editor-in-Chief* corrects only PDF editorial/conversion and index entry errors; corrections of all other errors are listed in the Errata of a subsequent issue for no charge. Authors should arrange payment of page charges and optional open access fees with the *Business Manager* <subscriptions@mycotaxon.com> at this time.

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A new host for *Puccinia menthae*

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Abstract—*Cyclotrichium* (*Lamiaceae*) is reported as a new host genus for the rust fungus, *Puccinia menthae.* The morphological and microscopical characteristics of this fungus are described and illustrated based on the collected materials.

Key words—Malatya, *Pucciniales*, taxonomy, Turkey

Introduction

The genus *Cyclotrichium* (Boiss.) Maden. & Scheng. (*Lamiaceae*, tribe *Mentheae*) contains nine species distributed in Turkey, Lebanon, Iraq, and Iran. Six *Cyclotrichium* species have been reported in Turkey, including two endemic species: *C. glabrescens* (Boiss. ex Rech.f.) Leblebici and *C. niveum* (Dirmenci et al. 2010, Dirmenci 2012). To our knowledge no rust fungi have previously been described on *Cyclotrichium* or cited for earlier synonyms of the species.

Puccinia menthae is an autoecious, macrocyclic, long-cycle rust recorded on 28 genera of *Lamiaceae* (Farr & Rossman 2015).

In Turkey, *Puccinia menthae* has been reported on *Calamintha*, *Clinopodium*, *Mentha*, *Micromeria*, *Origanum*, and *Satureja* (Bahçecioğlu & Kabaktepe 2012).

Here we present a record of *Puccinia menthae* on a new host genus, *Cyclotrichium*, from Malatya province, Turkey.

Materials & methods

The material on which this study is based was collected from Malatya in 2015. The host specimen was prepared according to established herbarium techniques. The host plant was identified according to Leblebici (1982) and Dirmenci et al. (2010). Spores

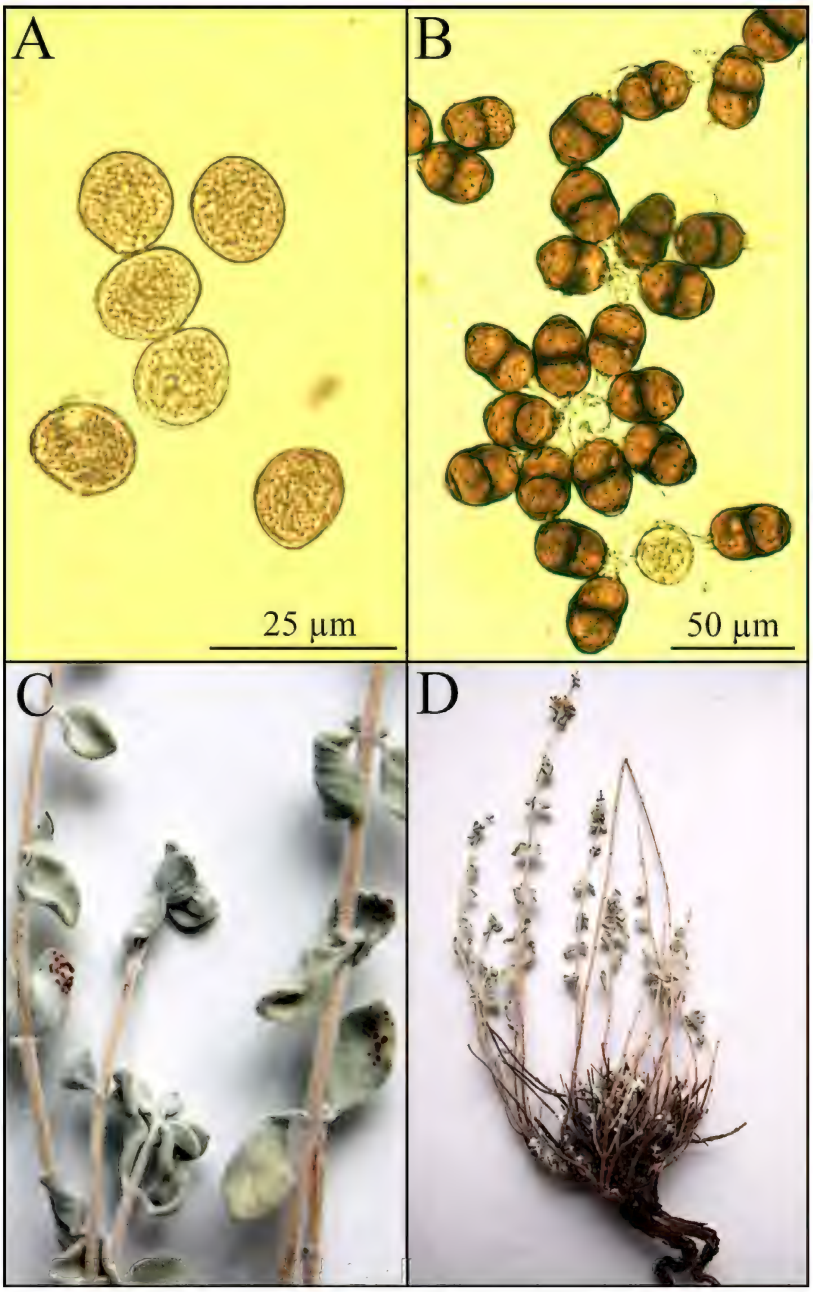

FIG. 1. *Puccinia menthae* on *Cyclotrichium niveum*. A, urediniospores; B, teliospores; C, infected brown areas on leaf tissue; D, herbarium specimen (INU 1200).

were scraped from the dried host specimen, mounted in lactophenol, and measured using Analysis LS Starter software.

Names and families of plants are given according to http://www.theplantlist.org. The specimen is preserved in the Inonu University Herbarium, Battalgazi, Malatya, Turkey (INU).

Taxonomy

Puccinia menthae Pers., Syn. Meth. Fung.: 227 (1801). FIG. 1

Spermogonia and aecia not found. Uredinia hypophyllous, rounded, scattered, 0.5–1 mm, surrounded by the epidermis, sometimes confluent, yellowish or brownish. Urediniospores globose to obovoid or ellipsoid, 17–28 × 14–19 µm; wall ≤2.5 µm thick, echinulate, hyaline to light yellow; germ pores 2, equatorial. Telia mostly hypophyllous, rarely amphigenous, on leaves, petioles and stems, rounded, blackish brown, scattered, pulverulent, 0.5–1.5 mm. Teliospores ellipsoid to obovoid, rounded at both ends, not or only slightly constricted at the septum, 26–35 × 19–23 µm, verrucose (sometimes smooth), brown, wall 1.5–3 µm thick at sides, 3–5 µm thick apically, apical in upper cell and close to septum in lower cell, with a hyaline papilla; pedicel slender, hyaline, longer than the spores.

SPECIMEN EXAMINED—On *Cyclotrichium niveum* (Boiss.) Manden. & Scheng. (*Lamiaceae*). Turkey: Malatya, Konak, Beydağı, 1700–1750 m a.s.l., 6.Sep.2015, T. Kolaç 1054 (INU 1200).

REMARKS: Our record of *Puccinia menthae* on *Cyclotrichium niveum* increases the host list of this cosmopolitan rust species to include 29 lamiaceous genera.

Acknowledgments

We thank Prof. Dr. Sevda Kirbag and Prof. Dr. Hacer Bakir Sert for presubmission expert reviews.

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Mycelephas levisporus sp. nov. on submerged wood from a freshwater habitat in Brazil

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& Luís Fernando Pascholati Gusmão[1*]

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Abstract—A new species from submerged wood in Brazil, *Mycelephas levisporus,* is described and illustrated. The long conidiophores and smooth wide conidia with slightly constricted septa are the main characteristics that distinguish the new species from the type species.

Key words—hyphomycetes, aeroaquatic fungi, helicoidal, neotropic

Introduction

During a study of microfungi occurring on submerged plant debris in the semi-arid Northeast region of Brazil, an interesting species of *Mycelephas* R.F. Castañeda was collected on submerged wood.

Mycelephas (Gams et al. 2009) is a monotypic genus described from dead wood in Cuba (Castañeda-Ruiz 1984; as *Arnoldiella* nom illeg.); it differs from other helicoidal microfungi by bifurcate, almost cochleate conidia coiled in two different planes (Castañeda-Ruiz 1984; Goos 1987; Seifert et al. 2011). Despite these unique morphological characters, other species such as *Helicoma resinae* (Lindau) J.L. Crane & Schokn, which has mono- or polyblastic conidiogenous cells, could be confused with *Mycelephas* based on its conidia coiled 3–4.5 times in one plane. *Diplorhynchus* G. Arnaud (Arnaud 1952) and *Dichotomophthoropsis* M.B. Ellis (Ellis 1971) also share some similarities

with *Mycelephas*. *Diplorhynchus* has hyaline conidia coiled in one plane and bifurcated at the end, and due to these characters Goos (1987) considered that genus as congeneric with *Mycelephas*; as no other records for *Diplorhynchus* have been registered since Arnaud (1952), however, its taxonomic status remains uncertain. *Dichotomophthoropsis* can be distinguished by its tretic conidiogenous cells, lobed cells, and helicoid conidia that do not bifurcate (Ellis 1971).

Materials & methods

Samples of submerged, decayed wood were collected in streams of Cochó River in Piatã and placed in plastic bags. In the laboratory, wood pieces were washed and placed in moist chambers. Subsequently, the moist chambers were placed in a large polystyrene box with sterile water plus glycerol at 24°C for 30 days and were examined periodically or once a week (Castañeda-Ruiz 2005). Permanent slides were prepared in PVL and micrographs were obtained with an Olympus microscope BX 51 with bright field and Nomarski interference optics. The type specimen of the new species is deposited in the Herbarium of Universidade Estadual de Feira de Santana, Bahia, Brazil (HUEFS).

Taxonomy

Mycelephas levisporus Cantillo, J. Mena & Gusmão **sp. nov.** Fig. 1

MycoBank MB 815045

Differs from *Mycelephas robustus* by its longer conidiophores and smooth and wider conidia.

Type: Brazil. Bahia: Piatã, Chapada Diamantina, Cochó River, 13°00'S 41°51'W, on submerged wood. 3.IV.2014, coll. T. Cantillo (**Holotype**, HUEFS 216594; **isotype**, HUEFS 216641).

Etymology: Latin, *levis* + *sporus*,-referring to the smooth conidia surface.

Anamorphic fungi, hyphomycetes. Colonies on natural substrate pulvinate, granulate, yellow to yellowish brown, aggregated. Mycelium mostly immersed in the substrate, septate, branched, brown. Conidiophores solitary, simple or branched, straight to flexuous, septate, smooth, light brown, turning brown with age, 93–205 × 7.5–15 µm; 7.5–12.5 µm at the apex. Conidiogenous cells mono or polyblastic, integrated, terminal or intercalary, sympodial, cylindrical, with dark scars. Conidial secession schizolytic. Conidia solitary, dry, light brown to subhyaline, smooth, helicoidal, 6–8 septa in the main filament, slightly constricted at septa. The filament bifurcates in two branches coiled 2–3 times giving an almost cochleate appearance, 9–15 µm diam. Mature conidia 23–(38)42.5 µm in side view, 25–37.5 µm in frontal view. The basal cells of conidia are V-shaped with truncate base; sometimes darker than other cells, 8–15 µm long, 2–3 µm at the base, and 6–10 µm at the apex.

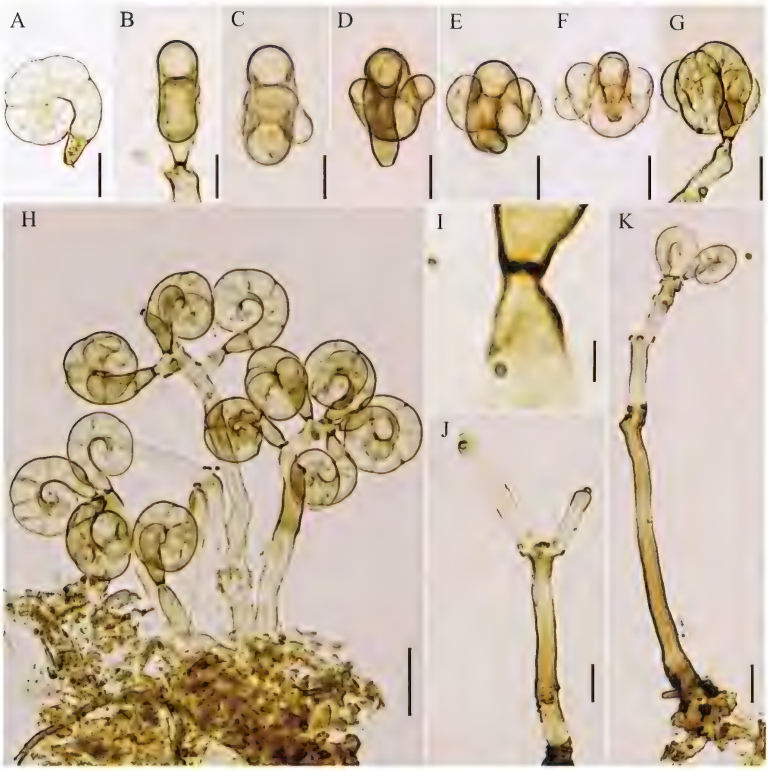

Fig. 1. *Mycelephas levisporus* (HUEFS 216594, holotype). A: immature conidium in side view; B–F: conidia in frontal view showing bifurcation of filament in two branches in different coiling stages; G: fully developed conidium; H: conidiophores and conidia; I: detail of conidiogenous cell; J, K: conidiophores showing integrated conidiogenous cells with dark scars. Scale bars: A–H, J = 10 µm; I = 2 µm; K = 20 µm.

Other material examined: *Mycelephas robustus* (R.F. Castañeda) R.F. Castañeda. Cuba. La Habana: Santiago de Las Vegas, on dead branches of *Pseudolmedia spuria* (Sw.) Griseb., 1.III.1983, coll. R.F. Castañeda (**Holotype**, C83/39 INIFAT).

Notes: *Mycelephas robustus* (R.F. Castañeda) R.F. Castañeda differs from *M. levisporus* by its smaller conidiophores (41–80 µm long) and its smaller rugose conidia (20–27 µm diam.; Castañeda-Ruiz 1984, as *Arnoldiella robusta*).

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Scutellospora tepuiensis sp. nov. from the highland tepuis of Venezuela

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Abstract—Examination of soil samples collected from the summit of Sororopán-tepui at La Gran Sabana, Venezuela, revealed an undescribed species of *Scutellospora* whose spores have an unusual and very complex ornamentation. The new species, named *Scutellospora tepuiensis*, is the fourth ornamented *Scutellospora* species described from La Gran Sabana and represents the first report of a glomeromycotan fungus for highland tepuis in the Venezuelan Guayana.

Key words—arbuscular mycorrhizal fungus, taxonomy, tropical species, *Gigasporaceae*, *Glomeromycetes*

Introduction

The Guayana shield occupies a vast area that extends approximately 1500 km in an east-to-west direction from the coast of Suriname to southwestern Venezuela and adjacent Colombia in South America. This region is mostly characterized by nutrient-poor soils and a flora of notable species richness, high endemism, and diversity of growth forms (Huber 1995). Vast expanses of hard rock (Precambrian quartzite and sandstone) that once covered this area as part of Gondwanaland (Schubert & Huber 1989) have been heavily weathered and fragmented by over a billion years of erosion cycles, leaving behind just a few strikingly isolated mountains (Huber 1995). These table mountains, with their sheer vertical walls and mostly flat summits, are the outstanding physiographic feature of the Venezuelan Guayana (Schubert & Huber 1989). The Pemón Amerindians of southeastern Venezuela call these mountains

"tepuis" (Huber 1995). A tepui is a sandstone mountain or tableland with a flat summit and vertical walls up to 600–3000 m tall. Rising abruptly over the forests and savannas of La Gran Sabana, Venezuela, these tepuis are quite isolated by their peculiar shape and harbor a high degree of endemic flora and fauna. Soils of tepuis are acid and extremely oligotrophic compared with their adjacent lowlands.

The often-steep tepui slopes are covered with dense mossy forests bathed in moisture from dense clouds that form along the cliffs. Many tepui summits have relatively little soil that is exposed to temperatures averaging 18–24 °C and heavy rainfall above 2000 mm. Dense clouds and prolonged mist are common and provide additional moisture at highland sites.

Arbuscular mycorrhizal fungi (AMF) are widespread in most terrestrial ecosystems where they form mutualistic associations with most plants and facilitate nutrient uptake from the soil via an extensive extraradical mycelium (Smith & Read 2008). This mycorrhizal association, which has a low degree of specificity between partners, involves a plant host and a fungus of the phylum *Glomeromycota* (Brundrett 2009, Smith et al. 2011). Although the fungi contribute many effects that may increase host plant survival and fitness, the presumed primary benefit to the plant is improved nutrition in exchange for carbohydrates to the fungal partner (Corrêa et al. 2014).

Previous works have reported an important role for glomeromycotan fungi in all lowland ecosystems of Venezuelan Guayana (Cuenca et al. 2003a,b), and three *Scutellospora* species have been described from the lowlands: *S. spinosissima* (Walker et al. 1998), *S. crenulata* (Herrera-Peraza et al. 2001), and *S. striata* (Cuenca & Herrera-Peraza 2008). However, the presence of this fungal group on tepui summits has remained unexplored.

During two brief trips to the Sororopán-tepui in Venezuelan Guayana, we found scutellosporoid spores with a very complex ornamentation representing an undescribed species. The fungus, described here as *Scutellospora tepuiensis*, constitutes the first report of an arbuscular mycorrhizal fungus for the highland tepuis.

Materials & methods

The 2050 m high Sororopán-tepui is part of the Ptari massif, a small mountain system lying north of La Gran Sabana at 5°40–50′N 61°40–50′W, where annual precipitation exceeds 2000 mm (Huber 1995). During two short trips made by helicopter to the summit of Sororopán-tepui, composite soil samples (15 subsamples taken 0–15 cm below ground level) were collected in two different tepui vegetation zones. Zone 1 was characterized by a low shrubland growing on acidic (pH 5.7), relatively deep soils rich (20%) in organic matter; the dominant vegetation (≤6 m tall) comprised woody species in the *Clusiaceae* and *Melastomataceae*. Zone 2 was characterized by pioneer vegetation growing between fissures and depressions of sandstone on shallow soil with

dominant species representing *Poaceae*, *Cyperaceae*, *Melastomataceae*, and *Orchidaceae;* zone 2 soils are also acidic (pH 5.9) but with less (16.5%) organic matter than in zone 1. We found our new *Scutellospora* species in both vegetation types accompanied by *Scutellospora spinosissima* C. Walker & Cuenca, *Acaulospora morrowiae* Spain & N.C. Schenck, several *Glomus* species, and another undescribed *Scutellospora*.

Open pot trap cultures started from the soil samples were maintained in a glasshouse for three months and then dried for one week. Two pre-germinated *Vigna luteola* seeds (the host plant) were placed in 1-liter pot with undiluted field soil; the pot contents were stored at room temperature (≈20°C) for almost one year to break spore latency (Morton et al. 1993), after which the spores were isolated and used to start pure cultures. Because these last pure cultures were unsuccessful, the description of the species presented below is based only on spores isolated from trap cultures (IVIC-38) and field soils.

Spores were isolated from the trap pots or field soils by wet sieving, decanting, and sucrose centrifugation (Sieverding 1991). The isolated spores were suspended in water and illuminated with light from a quartz-iodine fibre-optic source. Their color was determined by comparison with a British color chart for fungi (Anon 1969). The specimens were mounted in polyvinyl alcohol lacto-glycerol (PVLG) or in PVLG mixed with Melzer's reagent (1:1, v/v). Wall description and terminology follow Walker (1983) and Walker & Vestberg (1998). Type material was deposited in the Venezuelan National Herbarium, Universidad Central de Venezuela, Caracas, Venezuela (VEN) and the Cuban National Herbarium, IES-CITMA, La Habana, Cuba (HAC).

Spore wall ornamentation and structure was also examined with scanning electron microscopy (SEM). After removing or crushing the outermost spore wall layer with fine tweezers under a dissecting microscope, we rinsed the spores in a phosphate buffer solution prior to fixing in 1% osmium tetroxide at 4 °C for 1 h. Fixed samples were dehydrated for 5 min in each dilution of an acetone series (20, 40, 70, 80, and 100%), dried at critical point with liquid CO_2, placed on aluminum metal holders, coated with 200 Å gold–palladium, and then carefully examined by SEM.

Taxonomy

Scutellospora tepuiensis Furrazola & Cuenca, **sp. nov.** FIGS 1–3

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Differs from all other *Scutellospora* spp. by its spores formed singly in soil and by the complex spore ornamentation comprising numerous annulate pores, each surrounded by a lip composed of either gemmae or columellae.

TYPE: Venezuela, La Gran Sabana Region, Sororopán tepui; isolated from the rhizospheric soil of sclerophyllous shrubland, and pioneer vegetation growing between fissures and depressions of sandstones on a shallow soil under *Poaceae* and *Cyperaceae,* isolation date December 1996 by Z. De Andrade (**Holotype:** VEN-438658 [De Andrade 114-slide 2]; **isotype:** HAC-G-VE01 [De Andrade 114-slide 5).

ETYMOLOGY: Latin *tepuiensis*, referring to the "tepui" tableland where the species was found.

SPORES formed singly in soil terminally (rarely laterally) on a bulbous base (sporophore); saffron (49) to orange (48) when young, becoming sienna (11)

to fulvous (12) when mature; generally globose or subglobose, 162–216 × 159–219 µm (mean 191 × 190 µm, n = 28), and containing oily globular drops 8–35 µm diam.

Spore wall arrangement A(EL$_o$M)B(MMM) or A(EL$_o$)B(M)C(MMM) (following Walker 1983), here referred to A(12$_o$3)B(456) or A(12$_o$)B(3)C(456), with layer 2$_o$ comprising two sub-layers: 2$_o$A and 2$_o$B.

Outermost layer (1) rarely observed; when present, hyaline, smooth, ≤1 µm thick, readily visible in younger spores but frequently detaching in older ones; considered evanescent as not commonly found in mature spores.

Layer 2$_o$ 7–10 µm thick, laminated, composed of two sub-layers (2$_o$A, 2$_o$B). Sub-layer 2$_o$A dark yellow, 5–7 µm thick, perforated on its upper surface with 1–5 µm rounded to irregular pores, further inward apparently annulated and completely filled by round to elliptical or irregular 1.5–7 µm wide annuli separated by 1–7 µm with the in-between surface slightly depressed; each annulus with ~1-µm thick lip surrounded by 10–20 rounded gemmae or elliptical to clavate columellae, 1–3 µm long and ~1 µm thick, extending radially around the annulus pore as though hanging down into the lips (pores and annuli best seen in surface view); inward from each gemma or columella tip arise yeast-like projections, seemingly composed of chains or clusters of tiny (much smaller than 0.5 µm) wall material granules, the chains and clusters brush-like to diffuse and lacking in structure; the granules sometimes fusing together closer to 2$_o$A or 2$_o$B and then enlarging to ~1 µm; annuli with suspended gemma/columellae and yeast-like granule chains resembling a shower. [The information above based on crushed spores, with sub-layers somehow separating and granules disaggregating; we presume that the granules stay more closely together in nature]. Sub-layer 2$_o$B concolorous with 2$_o$A, but seeming darker when superimposed over sub-laminae; 2–3 µm thick, uniformly covered with hyaline to yellow bacula, 1–1.5 µm long, ≤0.5 µm wide; in unbroken spores, the bacula of this lower sub-layer intermingling with the yeast-like granule chains from the upper sub-layer, generally taller when just under the annulus pore.

Layer 3 tightly adhering to 2$_o$B, rarely separating from it and forming a separate group (B); <1 µm thick, hyaline, membranous.

Innermost wall group comprising layers 4, 5 and 6.

Layer 4 hyaline, ca. 1µm thick; in crushed spores smooth, slightly wavy or with a granular surface resembling a "beaded" membrane; commonly tightly adhering to layer 5, but separating or shrinking to form waves under applied pressure when the innermost group (endospore) is separated.

Layer 5 hyaline; 1 µm thick, expanding to 9-30 µm (in PVLG) or to 2–5 µm (in PVLG-Melzer's reagent), thus revealing its amorphous nature; when

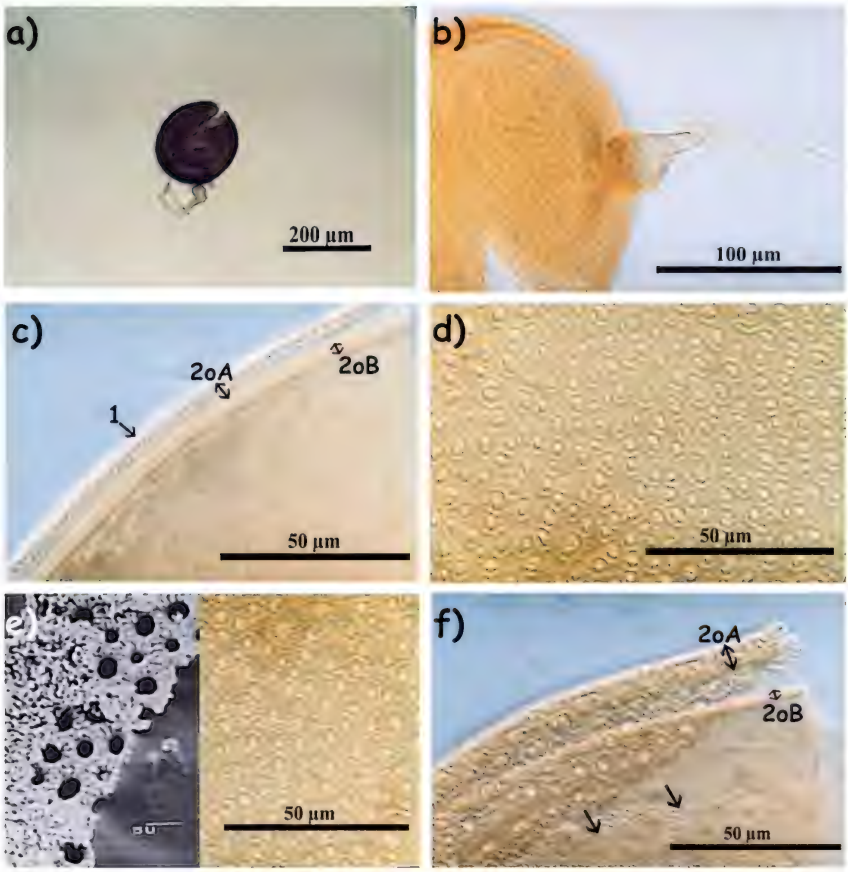

FIG. 1. *Scutellospora tepuiensis*. a) Whole spore mounted in water. b) Young spore crushed and mounted in PVLG. c) Crushed spore showing the external wall layer 1 and the two 2_0 layers. d) Surface view of layer 2 showing the ring-shaped ornamentation or annuli. e) Left: SEM image of the underside of layer 2_0A, showing gemmae radiating from the pores to form the "showers". Right: Underside (inner view) of the layer 2_0 surface, showing the rounded gemmae radiating from the annulus pores. f) Crushed spore showing the two sub-layers A and B that comprise layer 2_0. See the bacula of 2_0 B (arrowed) at lower right.

mounted in water, flexible and membranous and difficult to observe in the endospore wall.

LAYER 6 (innermost) a thin (<1 µm thick) flexible membrane that wrinkles considerably after crushing when mounted in PVLG but not in water or when intact.

In Melzer's layers 5 and 6 becoming red-purple while layer 2_0 turning deep yellow with the spore content partly yellow; after 24 hours the color reaction

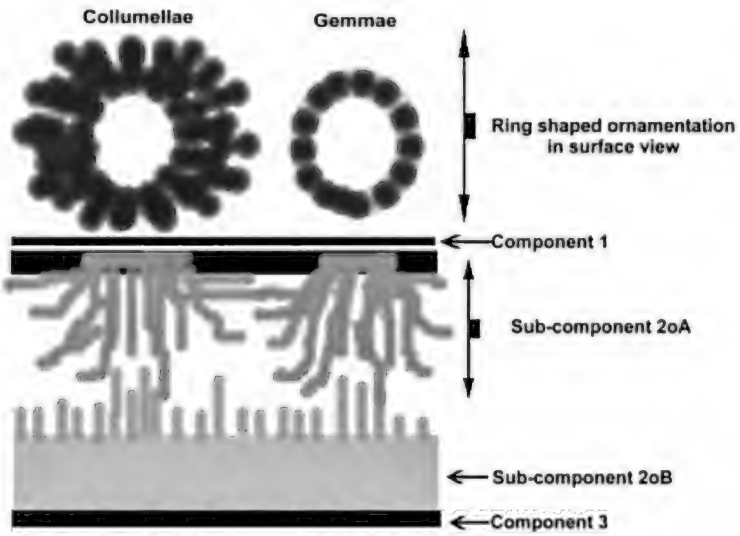

FIG. 2. Schematic representation of outermost wall layers of *Scutellospora tepuiensis*. Annular lips are defined by either columellae (at left) or gemmae (at right) radiating from a pore.

beginning to fade and after 3–4 days layer 5 turning pink to light purple and layer 6 remaining red-purple. Other layers do not react.

SPOROPHORE bulbous, 27–43 µm diam., 40–57 µm tall (from spore base to the subtending hypha septum); wall ornamented or smooth, contiguous with spore layers 1 and 2, thickness reaching 4–6 µm (≤9 µm near spore base), with one stout (10–35 µm) projection connected to a slender hypha.

GERMINATION SHIELD (on layer 4) broadly lobed, simple, hyaline, 87–110 × 30–65 µm, thin (<1 µm thick) walled.

AUXILIARY CELLS: unknown.

DISTRIBUTION & ECOLOGY—Known only from the summit of Sororopán-tepui, La Gran Sabana, Venezuela. Spores have been collected only from the low high-tepui shrubland and from the saxicolous community growing on sandstone at the summit. Soils are acidic and very low in exchangeable P (0.78–1.8 ppm).

MYCORRHIZAL ASSOCIATIONS unknown. Attempts to form mycorrhizas in pure culture failed, although the species sporulated abundantly in a multispecies pot culture with *Vigna luteola* as host.

ADDITIONAL COLLECTIONS EXAMINED: VENEZUELA, LA GRAN SABANA REGION, **Sororopán-tepui,** sclerophyllous shrubland, Dec. 1996, Z. De Andrade, De Andrade-110

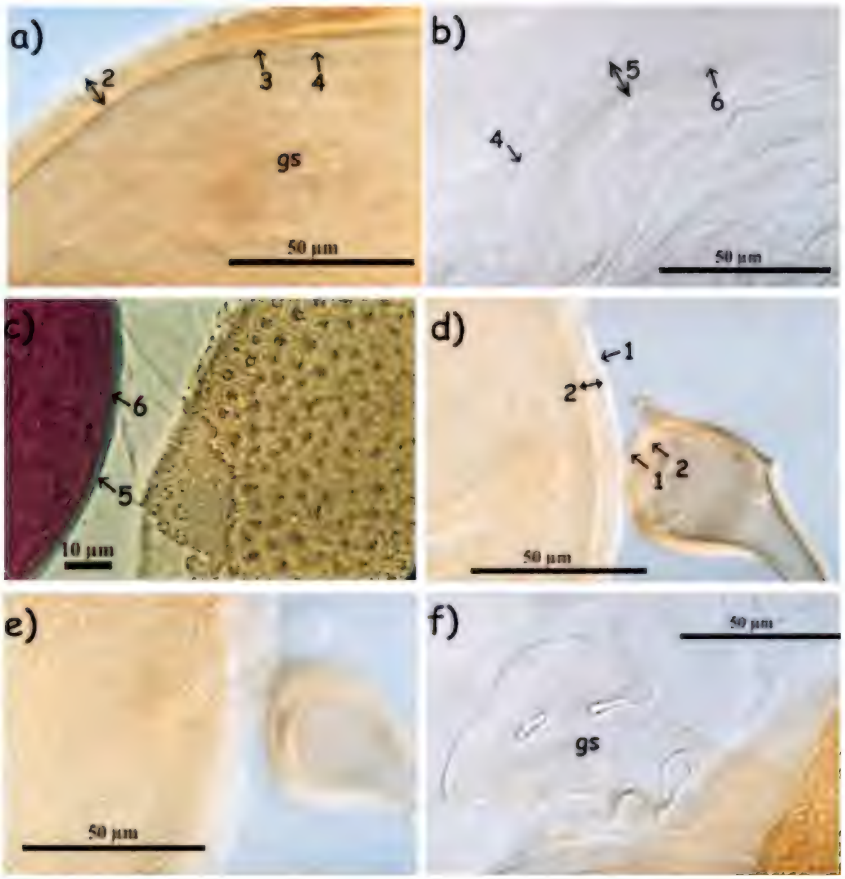

FIG. 3. *Scutellospora tepuiensis.* a) Crushed spore showing germination shield (gs) in lateral view. b) Crushed spore showing the inner group (4–6) of membranous layers and illustrating the amorphous nature of layer 5. c) Crushed spore showing the Melzer's reaction in layers 5 and 6. d) Bulbous suspensor showing its two layers contiguous with the spore layers. e) Ornamented bulbous suspensor. The ornamentation is not present in all specimens. f) Partial view of the germination shield.

(De Andrade personal herbarium); undiluted soil, trap culture (IVIC-38) with *Vigna luteola* as host plant, Dec. 1996, Z. De Andrade, De Andrade-118 (De Andrade personal herbarium); pioneer vegetation, Dec. 1996, De Andrade-123-(1/4) (De Andrade personal herbarium).

Discussion

The taxonomy and systematics of AMF species forming a bulbous suspensor and differentiating germinal walls (scutellosporoid spores) have changed dramatically over recent years. Oehl et al. (2008), who revised *Scutellospora*

based on morphological and genetic characters, split the genus into five genera and three families by weighting germination shield attributes as a diagnostic character. However, there are overlaps in germination shield morphology among these segregate genera. Analyses using different molecular tools and cladistics of 23 morphological characters by Morton & Msiska (2010) did not support most genera and families of Oehl et al. (2008) but indicated instead that species producing spores with a bulbous suspensor belong in a single family, *Gigasporaceae*, with only three genera: *Scutellospora*, *Gigaspora*, and *Racocetra*. More recently Redecker et al. (2013), who revised the classification of arbuscular mycorrhizal fungi—particularly the new taxa proposed by Oehl et al (2008)—accepted *Dentiscutata* and provisionally accepted *Cetraspora* (pending further study) but rejected *Quatunica, Intraornatospora*, and *Paradentiscutata* and retained all taxa of uncertain affinity in *Scutellospora*.

For *S. tepuiensis*, we were unable to see the germination shield in plane view as required by Oehl et al. (2008) for generic identification, although what we did see indicates that it is broadly lobed, a trait shared by at least two genera, *Cetraspora* and *Racocetra*. In addition, *S. tepuiensis* spores comprise six layers (or components) arranged in two or three groups, as found in most new genera erected by Oehl et al. (2008). Only *Racocetra* spores have one inner group of layers called a germinal wall (Morton & Msiska 2010). In the absence of molecular support for our new species and in view of its relatively simple germination shield, we prefer to adopt a conservative approach by describing this species in *Scutellospora*.

Scutellospora tepuiensis can be distinguished from other scutellosporoid species by its extremely complex ornamentation. Only *Dentiscutata reticulata* (Koske et al.) Sieverd. et al. has an ornamentation of such complexity. In addition, mature spores of both species are similar in color. However, S. *tepuiensis* spores have an annulate ornamentation, while *D. reticulata* spores are covered with a reticulum overlaying spines. *Dentiscutata nigra* has rounded holes in the outer wall, but its spores are much larger (≈500 µm) and darker-colored (dark brown to black) than those of *S. tepuiensis*. Other scutellosporoid species with a remarkable ornamentation are *S. crenulata* R.A. Herrera et al. (with spores covered with dome-like papillae) and *S. striata* Cuenca & R.A. Herrera (with spore ornamentation resembling a fingerprint). The other ornamented species retained in *Scutellospora* after the papers by Morton and Msiska (2010) and Redecker et al. (2013) are *S. nodosa* Błaszk. (with the outermost layer forming a knobbed spore surface) and *S. spinosissima* (with the laminate spore wall layer 2 ornamented with spines).

Three inner layers similar to those of *S. tepuiensis* have also been noted in other *Scutellospora* species, e.g., *S. spinosissima* (Walker et al. 1998),

S. projecturata Kramad. & C. Walker (Kramadibrata et al. 2000), *S. striata* (Cuenca & Herrera-Peraza 2008), and *S. crenulata* (Herrera-Peraza et al. 2001). According to Walker et al. (1998), the morphological features of the innermost wall layers of this fungal group probably differ from germinal wall 2 of other scutellosporoid species. In addition the layers forming this inner group frequently tightly adhere to each other and thereby this wall may resemble a structure consisting of one coriaceous layer (Walker et al. 1998).

Layer 4 of the inner wall group B or C of *S. tepuiensis* spores occasionally resembles a beaded layer commonly occurring in the innermost spore wall in species of *Acaulosporaceae*. A similar layer was also identified in spores of *S. crenulata* described from specimens found in La Gran Sabana (Herrera-Peraza et al. 2001). Molecular and cladistic morphological analyses support *Gigasporales* as more closely related to *Acaulosporaceae* than to *Glomerales* (Morton & Benny 1990). The presence of a beaded layer in *S. crenulata* and *S. tepuiensis* additionally supports this finding.

Future sampling of *S. tepuiensis* at the summit of Venezuelan tepuis will enable us to conduct the necessary molecular analysis that should indicate its phylogenetic position within *Scutellospora.* Nevertheless, its unique ornamentation when compared to all other described *Scutellospora* species distinguishes *S. tepuiensis* within *Gigasporaceae*. The La Gran Sabana ecosystem has become an important one for discovering new AMF species.

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Five penzigioid Xylaria species from Veracruz (Mexico)

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Abstract—Five species of the genus *Xylaria* with penzigioid stromata were collected from the state of Veracruz, Mexico. *Xylaria albocinctoides*, *X. discolor*, and *X. xylarioides* are new records for the Mexican mycobiota; *X. frustulosa* is a new record for Veracruz State; and *X. berteroi* is recorded from new localities within the state. Photographs of the stromata of the five species are presented, as well as a dichotomous key to the penzigioid *Xylaria* species known in Mexico.

Key words—*Ascomycota*, taxonomy, chorology

Introduction

Xylaria Hill ex Schrank is the type genus of the family *Xylariaceae*, with approximately 300 species (Kirk et al. 2008), some of which are characterized by penzigioid stromata. Penzigioid *Xylaria* were once placed in *Penzigia* Sacc., but Ju & Rogers (2001) showed that the type species, *P. cranioides* Sacc. & Paol., belongs to *Xylaria*. There are more than 40 penzigioid *Xylaria* species worldwide (Ju et al. 2012), characterized by sessile stromata and adherence to the substrate by a narrow central connective.

San Martín & Rogers (1995) recorded 105 *Xylaria* species in Mexico. Of the 109 species listed by Medel (2007), 45 are from Veracruz State (Medel et al. 2008). Penzigioid *Xylaria* species previously known from Mexico include *X. anisopleura* (Mont.) Fr., *X. berteroi*, *X. boergesenii* (Ferd. & Winge) P.F. Cannon, *X. enteroleuca* (J.H. Mill.) P.M.D. Martin, and *X. frustulosa* (San Martín & Rogers 1995). Chacón & Guzmán (1983) recorded "*Penzigia*

conostoma" (a name they attributed to J.H. Miller) from Veracruz, but Miller's (1961) "*Hypoxylon conostomum*" is a misdetermination of *X. boergesenii* (fide Cannon 1987).

The present study contributes to a better understanding of *Xylaria* species with penzigioid stromata from Veracruz State.

Materials & methods

We examined approximately 50 specimens of *Xylaria* with penzigioid stromata (all from localities in central Veracruz State), of which most were collected by the authors and a few are collections made by others and deposited at the Herbarium, Instituto de Ecología, A.C., Xalapa, Mexico (XAL). The stromata were sectioned and mounted in 5% KOH or Melzer's reagent. Descriptions are given for *X. albocinctoides*, *X. discolor*, and *X. xylarioides* (newly recorded from Mexico) and for *X. frustulosa,* not previously described based on Mexican material. Photographs of stromata are presented. All of the studied specimens were dried, labelled, and deposited at XAL, and duplicates were deposited at the Herbarium, Biodiversity Research Center, Academia Sinica, Taipei, Taiwan (HAST).

Taxonomy

Xylaria albocinctoides Y.M. Ju, H.M. Hsieh & J.D. Rogers, Mycologia 104: 768 (2012) PLATE 1A

STROMATA superficial, gregarious, aggregated to dispersed, sometimes confluent, semicircular, flattened to pulvinate, occasionally subglobose, reddish brown to blackish brown, slightly shiny, 0.5–2 mm diam × 0.5–0.8 mm thick, ≤10 mm long when confluent, attached to substrate by a narrow central connective, with 4–20 perithecia enclosed within each stroma, with white interior. PERITHECIA globose to subglobose, 0.2–0.3 mm diam. OSTIOLES black, papillate, 0.1 mm broad, slightly raised. ASCI with eight ascospores arranged in uniseriate manner, hyaline, cylindrical, 150–170 × 12–16 µm, with an amyloid apical ring. ASCOSPORES (18–)19–23 × 10–12 µm, dark brown to blackish brown, ellipsoid, inequilateral, somewhat rounded at ends, with a straight germ slit spore-length.

HABITAT—On dead branches of *Piper* sp. and secondary vegetation in a montane cloud forest at altitudes between 1300 and 1550 m.

SPECIMENS EXAMINED—**MEXICO, VERACRUZ STATE, Municipality of Xalapa,** Cerro Macuiltepec Ecological Park, 9 August 2011, S. Chacón, E. Utrera & F. Tapia 6204; 6 September 2011, S. Chacón & F. Tapia 6349; 27 September 2011, S. Chacón & F. Tapia 6387, 6388; 11 October 2011, S. Chacón & F. Tapia 6461; 25 October 2011, S. Chacón & F. Tapia 6533; 6 March 2013, S. Chacón & F. Tapia 6945; 10 April 2013, S. Chacón 7055-B; 11 July 2013, S. Chacón & F. Tapia 7207; 30 July 2013, S. Chacón & F. Tapia 7290; 5 August 2014, Chacón & F. Tapia 7699; INECOL Cloud Forest Sanctuary, Km 2.5

old road Xalapa–Coatepec, 25 August 2010, S. Chacón & E. Utrera 5929; 9 September, 2010, S. Chacón, E. Utrera & M. Soto 5970; 24 November 2010, S. Chacón & E. Utrera 6176; 22 August 2011, S. Chacón & E. Utrera 6246; 26 August 2011, S. Chacón, F. Tapia & E. Utrera 6292; 14 October 2011, S. Chacón 6477; 21 October 2011, S. Chacón & F. Tapia 6522; 28 October 2011, S. Chacón & F. Tapia 6546; 3 April 2014, F. Tapia 3008; 17 May 2012, S. Chacón & F. Tapia 6790-B; Tecajetes Park, 3 July 2013, S. Chacón, F. Tapia & E. Utrera 7154; Francisco Javier Clavijero Ecological Park, Km 2.5 old road Xalapa–Coatepec, 8 December 2003, S. Chacón 5506.

Remarks—*Xylaria albocinctoides* is a penzigioid species with a thin, soft stromatal surface. Macroscopically it is similar to *X. albocincta* (Rehm) Y.M. Ju et al., which differs mainly by larger ascospores 27–32 × 12–14 µm. The Mexican *X. albocinctoides* specimens have ascospore measurements very similar to those given in Ju et al. [2012; (18–)19–21(–22.5) × 10–11.5 µm]. *Xylaria albocinctoides,* previously known only from Venezuela, represents a new record for Mexico and is the most frequently encountered penzigioid *Xylaria* species recorded from Veracruz.

Xylaria berteroi (Mont.) Cooke ex J.D. Rogers & Y.M. Ju, N. Amer. Fung. 7(9): 18 (2012) Plate 1B

= *Penzigia enteroleuca* J.H. Mill., Mycologia 32: 183 (1940)

Habitat—On dead wood of angiosperms in a montane cloud forest, in a coffee farm within a secondary vegetation at altitudes of 1300 to 1600 m.

Specimens examined—**MEXICO, Veracruz State, Municipality of Xalapa,** San Roque Hills, 8 August 2012, F. Tapia & S. Chacón 2615; INECOL Cloud Forest Sanctuary, Km 2.5 old road Xalapa–Coatepec, 3 September 2010, S. Chacón & E. Utrera 5948; 1 October 2010, S. Chacón & E. Utrera 6049; 14 October 2011, S. Chacón & F. Tapia 6473; 6 June 2013, S. Chacón 7103; 19 June 2013, S. Chacón & F. Tapia 7109, 7128; 2 July 2013, S. Chacón & F. Tapia 7147, 8 August 2013, S. Chacón, E. Utrera & F. Tapia 7342, 7349; Natura Park protected area, 8 October 2011, S. Chacón & F. Tapia 6502; 27 February 2013, S. Chacón 6930; 3 April 2013, S. Chacón 7008, 7009, 7012, 7015, 7021; 19 July 2013, S. Chacón & F. Tapia 7233; Francisco Javier Clavijero Ecological Park, Km 2.5 old road Xalapa–Coatepec, 12 May 1982, Brown 416; 8 June 1983, S. Chacón 1068; 12 December 1983, S. Chacón 1934; 7 May 1986, L. Montoya 530; 23 March 1987, R. Medel 329; Francisco Javier Clavijero Botanical Garden, Km 2.5 old road Xalapa–Coatepec, 17 July 1991, S. Chacón 4519; 9 August 1995, S. Chacón 4925; 14 June 2000, R. Medel 756; 11 September 2006, R. Medel 1315; 2 September 2011, S. Chacón & E. Utrera 6331; 2 July 2012, F. Tapia 2576; 24 October 2012, F. Tapia 2691; Around the child care house CONECALLI, Km 2.5 old road Xalapa–Coatepec, 3 December 1990, F. Tapia 431; Cerro Macuiltepec Ecological Park, 6 September 2011, S. Chacón 6349-A; 27 September 2011, S. Chacón & F. Tapia 6390; 6 March 2013, S. Chacón 6935, 6948; 10 April 2013, S. Chacón 7065; 11 July 2013, S. Chacón & F. Tapia 7204, 7208; 30 July 2013, S. Chacón & F. Tapia 7279, 7285. **Municipality of San Andrés Tlalnehuayocan,** Agüita Fría Ranch, 6 April 2007, R. Medel 1351. **Municipality of Acatlán**, top Acatlán Volcano, 2 October 2013, S. Chacón, F. Tapia & E. Utrera 7419. **Municipality of Coatepec,** about 1 km of the road Coatepec–El Grande, 23 October 2012, F. Tapia 2686-B.

Remarks—*Xylaria berteroi* can be diagnosed by its more or less pulvinate to discoid stromata (0.7–2.5 cm diam × 0.2–0.7 cm thick), a thick hard carbonaceous outer stromatal layer (sometimes cracked on the surface), and ascospores [(9–)12–13.5(–14) × (6–)6.5–7.5(–8) µm] with a straight germ slit running almost the entire ascospore length. Welden et al. (1979) and Medel & Chacón (1997) reported *X. berteroi* from Veracruz State, and our research extends its known distribution to other localities in central Veracruz. The specimen "Brown 416", cited by Chacón & Guzmán (1983) as *Penzigia enteroleuca,* corresponds to *X. berteroi;* these two species are difficult to separate—Callan & Rogers (1990) suggested that they are probably conspecific, and Rogers & Ju (2012) treated them as conspecific.

Xylaria discolor (Berk. & Broome) Y.M. Ju, H.M. Hsieh, J.D. Rogers & Jaklitsch, Mycologia 104: 772 (2012) Plate 1C,D

= *Penzigia bermudensis* J.H. Mill., Mycologia 32: 402 (1940)

Stromata erumpent to superficial, dispersed, gregarious to confluent, subglobose to pulvinate, sometimes flattened, whitish grey to dark grey due to the presence of a outer layer, which falls off with age, leaving the surface a dark brown to blackish brown, 0.3–2 mm diam. × 0.5 mm thick, ≤1.5 cm long when confluent, attached to the substrate by a narrow central connective, containing ≤25 perithecia per stroma; surface dotted with ostioles; interior soft, yellow, whitish towards the base. Perithecia globose to subglobose, 0.1–0.3 mm diam. Ostioles black, papillate, 0.1 mm broad, slightly raised. Asci with eight uniseriately arranged ascospores, hyaline, cylindrical, 90–140 × 8–10 µm, with an inamyloid apical ring. Ascospores dark brown to blackish brown, ellipsoid, equilateral, with rounded apices, (8–)9–12 × 5.5–7.5(–8) µm, with a straight, spore-length germ slit.

Habitat—On dead wood in a montane cloud forest and in secondary vegetation of an abandoned coffee plantation. Altitudes 1300 to 1450 m.

Specimens examined—**MEXICO, Veracruz State, Municipality of Xalapa,** Francisco Javier Clavijero Botanical Garden, Km 2.5 old road Xalapa–Coatepec, 7 October 2010, S. Chacón & E. Utrera 6076; Palo Verde pantheon of Xalapa, 21 March 2012, S. Chacón 6984; Cologne El Olmo, former hacienda Las Animas, 17 April 2013, S. Chacón 7092; Cerro Macuiltepec Ecological Park, 28 August 2014, S. Chacón & F. Tapia 7783.

Remarks—Macroscopically *X. discolor* is somewhat similar to *X. lechatii* Y.M. Ju et al., but the latter species has a white interior, an apical ring staining pale blue only at base in Melzer's iodine reagent and larger ascospores 12–15 × 6.5–8 µm. According to Ju et al. (2012) and Rogers & Ju (2012), *Penzigia bermudensis* is a synonym of *X. discolor*. Seaver & Waterston (1940) described *P. bermudensis* with ascospores of 8–10 × 6–8 µm; our measurement of the

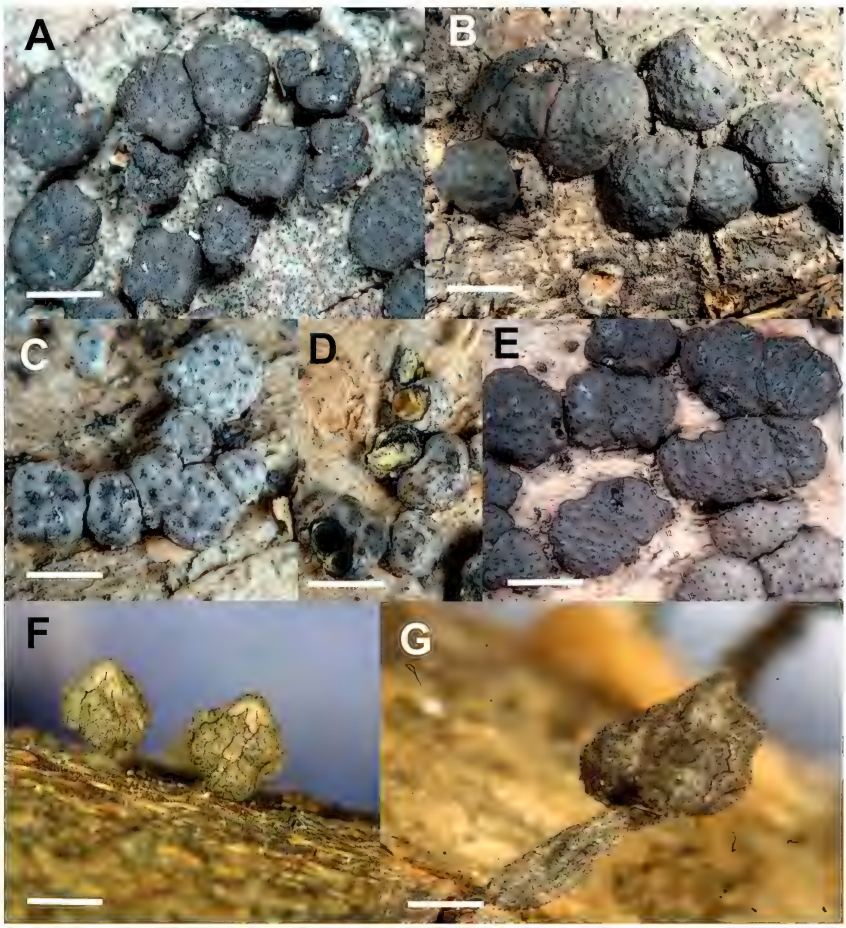

Plate 1. Stromata of *Xylaria* species. ***X. albocinctoides:*** A. scale bar = 2 mm. ***X. berteroi:*** B. scale bar = 7 mm. ***X. discolor:*** C. scale bar = 1.3 mm, D. scale bar = 1 mm. ***X. frustulosa:*** E. scale bar = 1.5 mm. ***X. xylarioides:*** F. scale bar = 1 mm, G. scale bar = 0.7 mm.

Mexican *X. discolor* ascospores agrees with those in Ju et al. (2012); (8.5–)9–12 × 5.5–7(–7.5) µm and Rogers & Ju (2012; 9–10.5 × 6–8 x 5–6 µm). This is the first record of *X. discolor* from Mexico.

Xylaria frustulosa Y.M. Ju, H.M. Hsieh & J.D. Rogers, (Berk. & M.A. Curtis) Cooke, Grevillea 12: 5 (1883) Plate 1E

≡ *Sarawakus frustulosus* (Berk. & M.A. Curtis) Lar. N. Vasiljeva, Nizshie Rasteniya, Griby i Mokhoobraznye Dalnego Vostoka Rossii 4: 157 (1998)

Stromata superficial, gregarious to confluent, semicircular to irregular, flattened to pulvinate, dark brown to black-brown, 0.5–2.7 mm diam. × 0.5–0.8 mm thick, ≤8 mm long when confluent, attached to the substrate by a narrow central connective, containing numerous perithecia; surface dotted with ostioles; interior soft, white. Perithecia globose to subglobose, 0.1–0.2 mm diam. Ostioles black, papillate, 0.1 mm wide, slightly raised. Asci with eight ascospores, hyaline, cylindrical, 40–50 × 4–6 µm, with a long stipe, and a small amyloid apical ring. Ascospores pale greenish brown, elliptical, 5–6(–7) × 2–3 µm, with an inconspicuous germ slit.

Habitat—On decorticated wood in a *Mangifera indica* plantation, alt. 500 m.

Specimen examined—**MEXICO, Veracruz State, Municipality of Jalcomulco,** beside restaurant El Rey, road Tlaltetela–Xalapa, 6 October 2012, S. Chacón 6869-B.

Remarks—*Xylaria frustulosa* is characterized mainly by its subdiscoid confluent stromata with small (no more than 0.8 mm) diameters and small ascospores with barely discernible germ slits. The ascospore size range of the Mexican material in general agrees that given by Miller (1934), Dennis (1970), Martin (1970), Jong & Rogers (1970), and Ju et al. (2012). The combination of this species in *Sarawakus* Lloyd (Vasilyeva 1998) is untenable; *Sarawakus* has a hypocrealean type species and is considered to be a synonym of *Trichoderma* (Samuels & Rossman 1992, Jaklitsch et al. 2014).

Xylaria frustulosa is known from tropical and subtropical regions in the Americas and Asia. San Martín & Rogers (1996) cited *X. frustulosa* (without a description) from Tamaulipas State, Mexico. Our collection represents the first record of *X. frustulosa* from Veracruz State.

Xylaria xylarioides (Speg.) Hladki & A.I. Romero,
Fungal Diversity 42: 86 (2010) Plate 1F, G

Stromata solitary to gregarious, the fertile part conical to subrhomboid or subcylindrical, 0.8–3 × 0.6–2 mm, with perithecial contours well pronounced and with the apex conical and sterile, sessile or sometimes with a short stipe ≤1.5 mm long, tomentose, concolorous with the fertile part; peeling layer arranged in pale brown bands, running from apex to middle. Perithecia globose to subglobose, 0.2–0.7 mm diam. Ostioles shiny black, papillate, small, up to 0.1 mm diam; surface roughened, black to blackish brown; interior soft, white. Asci containing eight uniseriately arranged ascospores, hyaline, cylindrical, 140–195 × 8–9 µm, with an amyloid apical ring. Ascospores brown to blackish brown, ellipsoid to navicular, inequilateral, with apices more or less rounded or mucronate, (17–)19–22 × (6.5–)7–9.5 µm, with a straight germ slit running almost the entire spore length.

Habitat—On dead branches of unidentified angiosperms, in a montane cloud forest and natural secondary vegetation at altitudes of 1300 to 1600 m.

Specimens examined—**MEXICO, Veracruz State, Municipality of Xalapa,** Cerro Macuiltepec Ecological Park, 11 October 2011, S. Chacón & F. Tapia 6459; 11 July 2013, S. Chacón 7199; INECOL Cloud Forest Sanctuary, Km 2.5 old road Xalapa–Coatepec, 30 September 2011, S. Chacón & E. Utrera 6419; S. Chacón & F. Tapia 6420; Tecajetes Park, 1 August 2013, S. Chacón, E. Utrera & F. Tapia 7305; Francisco Javier Clavijero Ecological Park, Km 2.5 old road Xalapa–Coatepec, 24 April 2014, F. Tapia & S. Chacón 3023.

Remarks—This species is characterized by the presence of a conical sterile apex, a well–developed peeling layer, and ascospore size and shape. The Mexican material agrees with the description of Hladki & Romero (2010; ascospores 17–21 × 6.5–9 µm). The Mexican material of *X. xylarioides* shows a great variability in stipe length; in some collections the stipe is almost indiscernible but in others it reaches up to 3 mm long.

Dichotomous key to penzigioid *Xylaria* species in Mexico

1. Apex of the stromata conical, sterile; ascospores 17–22 × 6.5–9.5 µm ..... *X. xylarioides*
1. Apex of stromata flattened or convex, fertile 2
2. Ascospores >20 µm long 3
2. Ascospores <20 µm long 4
3. Stromata flattened to pulvinate 6
3. Stromata subglobose to elongate, 0.4–4 cm high; ascospores 20–30 × 7.5–9.5 µm ..... *X. anisopleura*
4. Stromata 1–2 cm diam, with a carbonaceous outer layer; ascospores 9–14 × 6–8 µm ..... *X. berteroi*
4. Stromata 0.5 mm diam, with a thin and soft outer layer 5
5. Stromatal interior yellow; ascospores 8–12 × 5.5–8 µm ..... *X. discolor*
5. Stromatal interior white; ascospores 5–7 × 2–3 µm ..... *X. frustulosa*
6. Ascospores 18–23 × 10–12 µm, with a straight germ slit ..... *X. albocinctoides*
6. Ascospores 19.5–26 × 7–8.5 µm, with a spiral to sigmoid germ slit ... *X. boergesenii*

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Three species of wood-decaying fungi in *Polyporales* new to China

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Abstract—Three wood-decaying fungi, *Ceriporiopsis lagerheimii*, *Sebipora aquosa*, and *Tyromyces xuchilensis,* are newly recorded in China. The identifications were based on morphological and molecular evidence. The phylogenetic tree inferred from ITS+nLSU sequences of 49 species of *Polyporales* nests *C. lagerheimii* within the phlebioid clade, *S. aquosa* within the gelatoporia clade, and *T. xuchilensis* within the residual polyporoid clade. The three species are described and illustrated based on Chinese material.

Key words—*Basidiomycota*, polypore, taxonomy, white rot fungus

Introduction

Wood-decaying fungi play a key role in recycling nutrients of forest ecosystems by decomposing cellulose, hemicellulose, and lignin of the plant cell walls (Floudas et al. 2015). *Polyporales*, a large order in *Basidiomycota*, includes many important genera of wood-decaying fungi. Recent molecular studies employing multi-gene datasets have helped to provide a phylogenetic overview of *Polyporales*, in which thirty-four valid families are now recognized (Binder et al. 2013).

The diversity of wood-decaying fungi is very high in China because of the large landscape ranging from boreal to tropical zones. More than 1200 species of wood-decaying fungi have been found in China (Dai 2011, 2012), and some

[a] Chang-lin Zhao and Shi-liang Liu contributed equally to this work and share first-author status

important genera have been extensively investigated (Jia & Cui 2011; He & Dai 2012; Zhou & Dai 2012, 2013; Li & Cui 2013; Li et al. 2014; Song et al. 2014; Chen et al. 2015, 2016; Zhou 2015; Zhou et al. 2015, 2016a,b,c). Recently, three additional species in *Polyporales*—*Ceriporiopsis lagerheimii*, *Sebipora aquosa*, and *Tyromyces xuchilensis*—were found in Yunnan Province, southwestern China, and are described and illustrated here. In addition to morphological analysis, the phylogenetic positions of these Chinese specimens were inferred from ITS+nLSU sequences.

Materials & methods

Morphological study

The studied specimens are deposited at the herbaria of Beijing Forestry University, Beijing, China (BJFC) and University of Oslo, Oslo, Norway (O). Macro-morphological descriptions are based on field notes. Special colour terms follow Petersen (1996). Micro-morphological data were obtained from the dried specimens under a light microscope. The following abbreviations are used: KOH = 5% potassium hydroxide, CB = Cotton Blue, CB– = acyanophilous, IKI = Melzer's reagent, IKI– = neither amyloid nor dextrinoid, L = mean spore length, W = mean spore width, Q = variation in the L/W ratios, n = number of spores (a) measured from number of specimens (b).

Molecular phylogeny

A CTAB rapid plant genome extraction kit-DN14 (Aidlab Biotechnologies Co., Ltd, Beijing) was used to obtain PCR products from dried specimens, according to the manufacturer's instructions with some modifications. ITS region was amplified with the primer pair ITS5 and ITS4 (White et al. 1990), and the nuclear LSU region was amplified with the primer pair LR0R and LR7 (http://www.biology.duke.edu/fungi/mycolab/primers.htm). The ITS PCR began with initial denaturation at 95°C for 3 min, followed by 35 cycles at 94°C for 40 s, 58°C for 45 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. The nLSU PCR began with initial denaturation at 94°C for 1 min, followed by 35 cycles at 94°C for 30 s, 48°C for 1 min, and 72°C for 1.5 min, and a final extension at 72°C for 10 min. The PCR products were purified and sequenced at Beijing Genomics Institute. The sequences were deposited at GenBank (Table 1).

Sequencher 4.6 (GeneCodes, Ann Arbor) was used to edit the DNA sequences. The original ITS and nLSU sequences were combined directly and then aligned in MAFFT 6 (Katoh & Toh 2008, http://mafft.cbrc.jp/alignment/server/) using the "G-INS-I" strategy and manually adjusted in BioEdit (Hall 1999). The concatenated alignment was subjected to the incongruence length difference (ILD) test (Farris et al. 1994) implemented in PAUP* 4.0b10 (Swofford 2002) with a heuristic search and 1000 bootstrap (BS) replicates. The ILD test generated a P value of 1.000 much greater than 0.01, indicating that there was no incongruence between the ITS and nLSU regions. The sequence alignment was deposited in TreeBase (submission ID 19179). *Heterobasidion annosum* (Fr.) Bref. and *Stereum hirsutum* (Willd.) Pers. were selected as outgroup (Binder et al. 2013). Clade names follow Binder et al. (2013).

TABLE 1. Species and sequences used in the phylogenetic analyses. Newly generated sequences are set in bold.

SPECIES	VOUCHER NO.	GENBANK NO.	
		ITS	nLSU
Abortiporus biennis (Bull.) Singer	TFRI 274	EU232187	EU232235
Antrodia albida (Fr.) Donk	CBS 308.82	DQ491414	AY515348
Antrodia heteromorpha (Fr.) Donk	CBS 200.91	DQ491415	AY515350
Antrodiella americana Ryvarden & Gilb.	Gothenburg 3161	JN710509	JN710509
Antrodiella semisupina (Berk. & M.A. Curtis) Ryvarden	FCUG 960	EU232182	EU232266
Ceriporiopsis balaenae Niemelä	H7002389	FJ496669	FJ496717
Ceriporiopsis consobrina (Bres.) Ryvarden	Rivoire 977	FJ496667	FJ496716
Ceriporiopsis fimbriata C.L. Zhao & Y.C. Dai	Dai 11672	KJ698633	KJ698637
	Cui 1671	KJ698634	KJ698638
Ceriporiopsis gilvescens	BRNM 667882	FJ496685	FJ496719
	BRNM 710166	FJ496684	FJ496720
	Yuan 2752	KF845946	KF845953
Ceriporiopsis guidella	HUBO 7659	FJ496687	FJ496722
Ceriporiopsis lagerheimii	**Ryvarden 58240**	**KX081077**	**KX161652**
	Dai 12304	**KX161647**	**KX161651**
Climacocystis borealis	KH 13318	JQ031126	JQ031126
Coriolopsis caperata (Berk.) Murrill	LE(BIN)-0677	AB158316	AB158316
Dacryobolus karstenii (Bres.) Oberw. ex Parmasto	KHL 11162	EU118624	EU118624
Daedalea quercina (L.) Pers.	DSM 4953	DQ491425	DQ491425
Earliella scabrosa (Pers.) Gilb. & Ryvarden	PR1209	JN165009	JN164793
Fomitopsis pinicola (Sw.) P. Karst.	CBS 221.39	DQ491405	DQ491405
Fomitopsis rosea (Alb. & Schwein.) P. Karst.	ATCC 76767	DQ491410	DQ491410
Fragiliporia fragilis Y.C. Dai et al.	Dai 13080	KJ734260	KJ734264
	Dai 13559	KJ734261	KJ734265
	Dai 13561	KJ734262	KJ734266
Ganoderma lingzhi Sheng H. Wu et al.	Wu 1006-38	JQ781858	—
Gelatoporia subvermispora	BRNU 592909	FJ496694	FJ496706
Grammothelopsis subtropica B.K. Cui & C.L. Zhao	Cui 9041	JQ845096	JQ845099
Heterobasidion annosum	PFC 5252	KC492906	KC492906
Hornodermoporus martius (Berk.) Teixeira	MUCL 41677	FJ411092	FJ393859

Table 1, concluded

Species	Voucher no.	GenBank no.	
		ITS	nLSU
Hypochnicium lyndoniae (D.A. Reid) Hjortstam	NL 041031	JX124704	JX124704
Junghuhnia nitida (Pers.) Ryvarden	KHL 11903	EU118638	EU118638
Obba rivulosa (Berk. & M.A. Curtis) Miettinen & Rajchenb.	KCTC 6892	FJ496693	FJ496710
Obba valdiviana (Rajchenb.) Miettinen & Rajchenb.	FF 503	HQ659235	HQ659235
Perenniporia medulla-panis (Jacq.) Donk	MUCL 49581	FJ411088	FJ393876
Perenniporiella neofulva (Lloyd) Decock & Ryvarden	MUCL 45091	FJ411080	FJ393852
Phlebia livida (Pers.) Bres.	FCUG 2189	AF141624	AF141624
Phlebia radiata Fr.	UBCF 19726	HQ604797	HQ604797
Phlebia subserialis (Bourdot & Galzin) Donk	FCUG 1434	AF141631	AF141631
Piloporia sajanensis (Parmasto) Niemelä	Mannine 2733a	HQ659239	HQ659239
Podoscypha venustula (Speg.) D.A. Reid	CBS 65684	JN649367	JN649367
Polyporus tuberaster (Jacq. ex Pers.) Fr.	CulTENN 8976	AF516598	AJ488116
Postia guttulata (Sacc.) Jülich	KHL 11739	EU11865	EU11865
Sebipora aquosa	Miettinen 8868	HQ659242	—
	Miettinen 8680	HQ659240	HQ659240
	Miettinen 9265	HQ659243	—
	Dai 13268	**KX161648**	**KX161661**
	Dai 13592	**KU376422**	**KX161660**
Skeletocutis amorpha (Fr.) Kotl. & Pouzar	Miettinen 11038	FN907913	FN907913
Skeletocutis jelicii Tortič & A. David	H 6002113	FJ496690	FJ496727
Skeletocutis portcrosensis A. David	LY 3493	FJ496689	FJ496689
Skeletocutis subsphaerospora A. David	Rivoire 1048	FJ496688	FJ496688
Steccherinum fimbriatum (Pers.) J. Erikss.	KHL 11905	EU118668	EU118668
Steccherinum ochraceum (Pers.) Gray	KHL 11902	JQ031130	JQ031130
Stereum hirsutum	NBRC 6520	AB733150	AB733325
Truncospora ochroleuca (Berk.) Pilát	MUCL 39726	FJ411098	FJ393865
Tyromyces chioneus (Fr.) P. Karst.	Cui 10225	KF698745	KF698756
Tyromyces xuchilensis	**Dai 12234**	**KX161649**	**KX161658**
	Ryvarden 44669	**KX161650**	**KX161659**
Xanthoporus syringae (Parmasto) Audet	Gothenburg 1488	JN710607	JN710607

Maximum parsimony analysis was applied to the ITS+nLSU sequences dataset, and performed in PAUP* version 4.0b10 (Swofford 2002) according to Zhao et al. (2013). All characters were equally weighted, and gaps were treated as missing data. Trees were inferred using the heuristic search option with TBR branch swapping and 1000 random sequence additions. Max-trees were set to 5000, branches of zero length were collapsed, and all parsimonious trees were saved. Clade robustness was assessed using a bootstrap (BT) analysis with 1000 replicates (Felsenstein 1985). Descriptive tree statistics, tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI) were calculated for each generated Maximum Parsimonious Tree (MPT). Sequences were also analyzed using Maximum likelihood (ML) with RAxML-HPC2 on Abe through the Cipres Science Gateway (www.phylo.org). Branch support for ML analysis was determined by 1000 bootstrap replicate.

MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004) was used to determine the best-fit evolution model for Bayesian inference (BI). BI was calculated with MrBayes3.1.2 with a general time reversible (GTR) model of DNA substitution and a gamma distribution rate variation across sites (Ronquist & Huelsenbeck 2003). Four Markov chains were run for 10 million generations, and trees were sampled every 100 generations. The first quarter generations were discarded as burn-in. A majority rule consensus tree of all remaining trees was calculated.

Results

Phylogenetic analyses

The combined dataset (ITS+nLSU) included sequences from 62 samples representing 51 species. The dataset had an aligned length of 2202 characters, of which 1294 characters are constant, 232 are variable and parsimony-uninformative, and 676 are parsimony-informative. MP analysis yielded 13 equally parsimonious trees (TL = 4893, CI = 0.313, HI = 0.687, RI = 0.578, RC = 0.181). Best model estimated and applied in the Bayesian analysis was: GTR+I+G, lset nst = 6, rates = invgamma; prset statefreqpr = dirichlet (1,1,1,1). The average standard deviation of split frequencies in BI was 0.001416. BI and ML analyses resulted in similar topologies as that of MP analysis.

The MP phylogenetic tree supported seven major clades for 49 species of *Polyporales* with *Ceriporiopsis lagerheimii* nested within the phlebioid clade, *Sebipora aquosa* within the gelatoporia clade, and *Tyromyces xuchilensis* within the residual polyporoid clade (Fig. 1). For all these three species, specimens from China clustered with authentic specimens from elsewhere with strong support (Fig. 1).

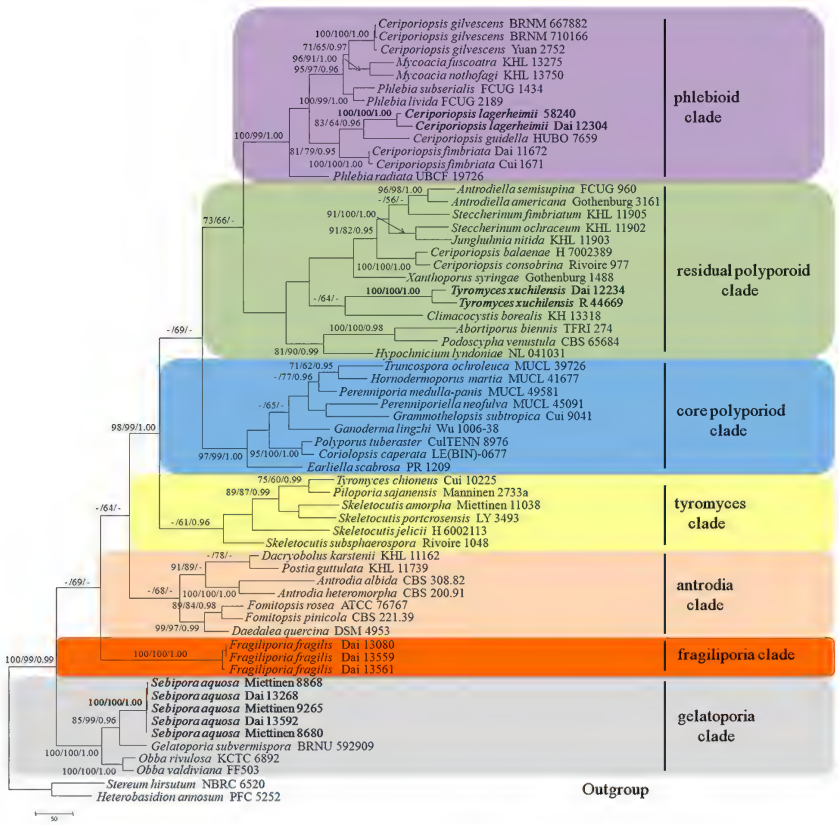

FIG. 1. Maximum parsimony strict consensus tree illustrating the phylogeny of *Ceriporiopsis lagerheimii*, *Sebipora aquosa*, *Tyromyces xuchilensis*, and related species in *Polyporales* based on ITS+nLSU sequences. Branches are labeled with maximum likelihood bootstrap ≥70%, parsimony bootstrap proportions ≥50% and Bayesian posterior probabilities ≥0.95.

Taxonomy

Ceriporiopsis lagerheimii Læssøe & Ryvarden, Syn. Fung. 27: 44, 2010. FIGS 2a, 3

BASIDIOME Annual, resupinate, adnate, soft corky, without odor or taste when fresh, corky when drying; ≤2 cm long, 1.5 cm wide, 2 mm thick at center. Pore surface cream when fresh, cream to buff upon when drying; pores angular, 5–6 per mm; dissepiments thin, entire. Subiculum thin, white, ≤0.2 mm thick. Tubes concolorous with pore surface, ≤1.8 mm long.

HYPHAL STRUCTURE Monomitic; generative hyphae with clamp connections; IKI–, CB–; tissues unchanged in KOH.

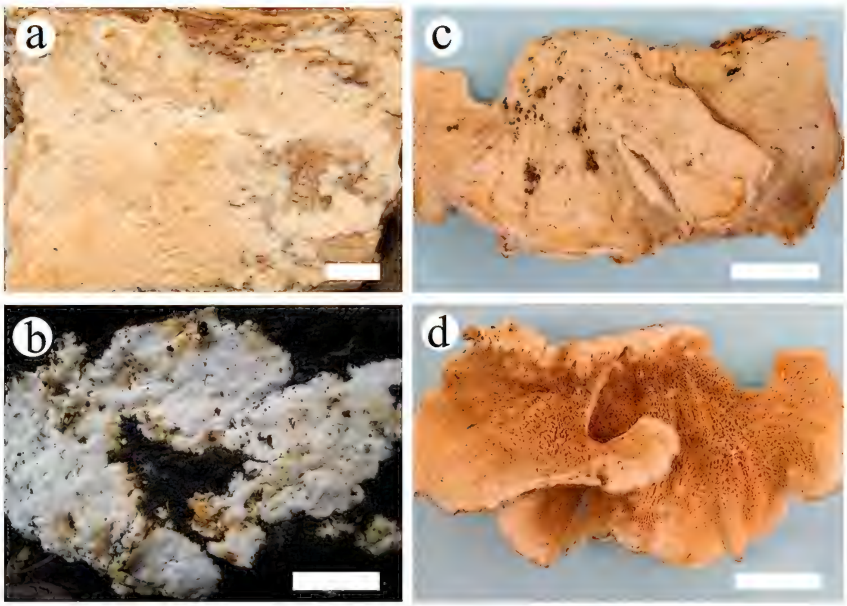

Fig. 2. Basidiocarps: a. *Ceriporiopsis lagerheimii* (Dai 12304); b. *Sebipora aquosa* (Dai 13592); c, d. *Tyromyces xuchilensis* (Dai 12234). Scale bars: a = 2 mm; b = 2 cm; c, d = 1 cm.

Subiculum Generative hyphae hyaline, thin-walled, branched, flexuous, interwoven, 3–5 μm in diam.

Tubes Generative hyphae hyaline, thin-walled, branched, flexuous, interwoven, 3.5–4.5 μm in diam. Cystidia and cystidioles absent. Basidia barrel-shaped to pyriform, hyaline, thin-walled, with four sterigmata and a basal clamp connection, 9–14 × 5–7 μm; basidioles similar to basidia, but slightly smaller.

Spores Basidiospores cylindrical, tapering toward apiculus, hyaline, thin-walled, smooth, IKI–, CB–, (3–)3.2–3.7(–4) × (1.3–)1.5–1.8(–2) μm, L = 3.5 μm, W = 1.7 μm, Q = 2.1 (n = 30/1).

Type of rot: White rot.

Specimens examined: **CHINA. Yunnan Province**: Jinghong, Xishuangbanna Nature Reserve, Sanchahe, on rotten angiosperm wood, 7 June 2011, Dai 12304 (BJFC010586). **ECUADOR. Napo Province**, Cuyuja, 4 May 2002, Ryvarden 58240 (isotype in O).

Comments: The Chinese specimen has cream to buff pore surface after drying, while the isotype specimen has white to cream pore surface. Otherwise, they have same morphological characters.

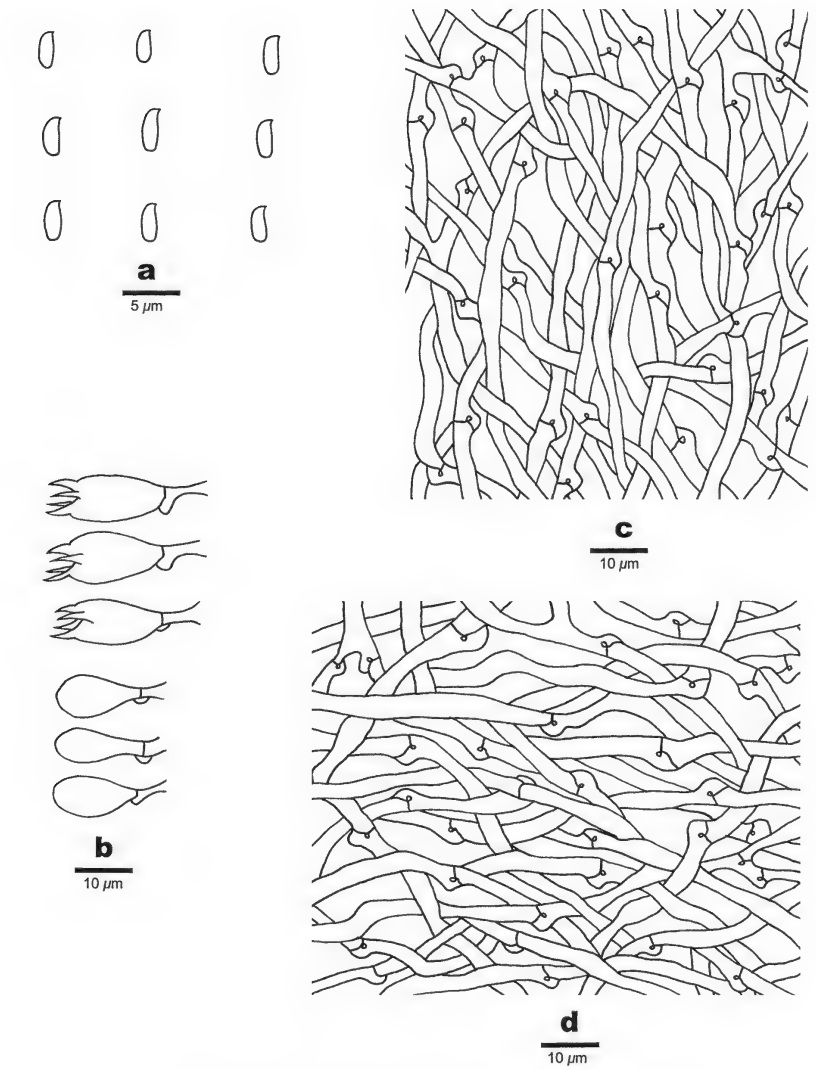

Fig. 3. *Ceriporiopsis lagerheimii* (drawn from Dai 12304).
a. Basidiospores; b. Basidia and basidioles; c. Tramal hyphae; d. Subicular hyphae.

Sebipora aquosa Miettinen, Mycol. Prog. 11: 144, 2012. Figs 2b, 4

Basidiome Annual, resupinate, watery and soft when fresh, becoming hard corky when drying; ≤9 cm long, 5 cm wide, 8 mm thick at center. Pore surface white when fresh, cream to yellow upon drying, more or less tallowing; pores

angular, 4–6 per mm; dissepiments thin, entire. Sterile margin cream to pale brown, ≤1 mm wide. Context white to cream, soft corky, ≤1 mm thick. Tubes concolorous with pore surface, hard corky, ≤7 mm long.

Hyphal structure Monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH.

Subiculum Generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, more or less parallel to substrate, 3–5 µm in diam.

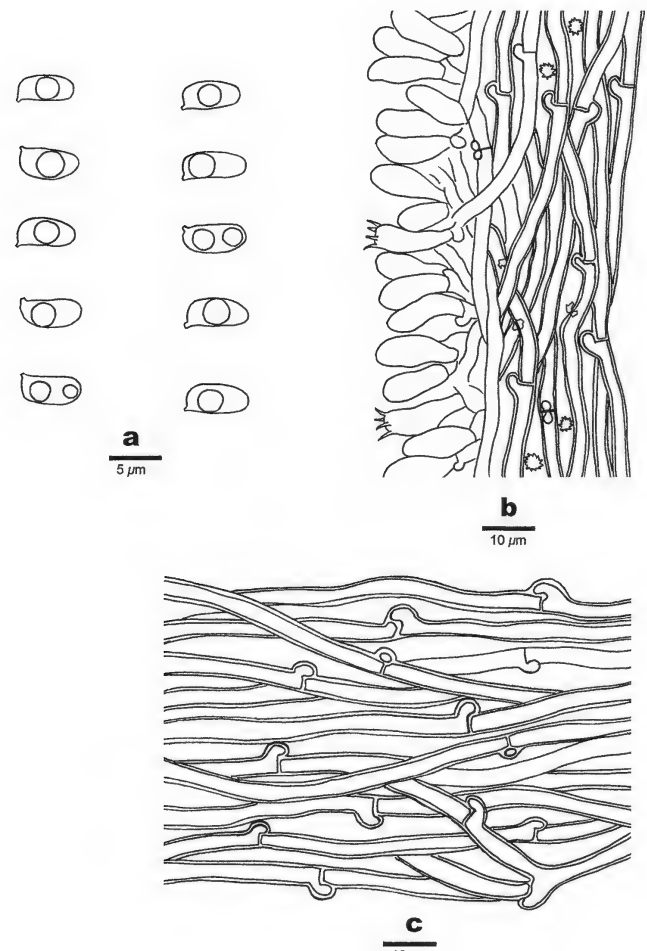

Fig. 4. *Sebipora aquosa* (drawn from Dai 13592).
a. Basidiospores; b. Tramal section; c. Subicular hyphae.

Tubes Generative hyphae hyaline, thin- to thick-walled, unbranched, subparallel to the tubes, 2.5–4 μm in diam. Small pale-yellow resinous granules occasionally present. Cystidia and cystidioles absent. Basidia clavate to pyriform, with four sterigmata and a basal clamp connection, 16–24 × 5–6 μm; basidioles similar to basidia, but slightly smaller.

Spores Basidiospores cylindrical to oblong-ellipsoid, often slightly curved, hyaline, thin-walled, smooth, usually bearing one or two guttules, IKI–, CB–, (5.5–)6–6.5(–6.7) × (2–)2.2–2.8 μm, L = 6.2 μm, W = 2.5 μm, Q = 2.3–2.5 (n = 60/2).

Type of rot: White rot.

Specimens examined: **CHINA. Yunnan Province**: Jinghong, Xishuangbannan Nature Reserve, Wangtianshu, on fallen angiosperm trunk, 19 October 2013, Dai 13592 (BJFC015054); Nanhua County, Dazhongshan Nature Reserve, on rotten angiosperm wood, 15 July 2013, Dai 13268 (BJFC014755).

Comments: The Chinese material conforms closely to the original description, which cites slightly longer basidiospores (5.7–7.6 × 2.1–2.7 μm; Miettinen & Rajchenberg 2012).

Tyromyces xuchilensis (Murrill) Ryvarden, Mycotaxon 23: 175, 1985. Figs 2c,d, 5

Basidiome Annual, pileate, soft and sappy when fresh, shrinking and becoming corky when drying. Pilei more or less semicircular, projecting ≤3 cm long, 4 cm wide, and 5 mm thick at center. Pileal surface white when fresh, becoming cream to pale ochraceous upon drying, glabrous. Pore surface white when fresh, buff to ochraceous upon drying; sterile margin cream, ≤1 mm wide; pores angular, 3–5 per mm; dissepiments thin, entire. Context white to cream, soft corky, ≤1 mm thick. Tubes concolorous with pore surface, corky, ≤4 mm long.

Hyphal structure Monomitic; generative hyphae with clamp connections, IKI–, CB–; tissues unchanged in KOH.

Context Generative hyphae hyaline, thin- to slightly thick-walled, occasionally branched, interwoven, 4–7 μm in diam.

Tubes Generative hyphae hyaline, thin- to slightly thick-walled, unbranched, interwoven, more or less parallel to tubes, 3–5 μm in diam. Cystidia and cystidioles absent; basidia clavate, with four sterigmata and a basal clamp connection, 15–18.5 × 5–7 μm; basidioles similar in shape to basidia, but slightly smaller.

Spores Basidiospores subglobose to broadly ellipsoid, hyaline, thin-walled, smooth, IKI–, CB–, (3.3–)3.5–4.4(–4.7) × (2.3–)2.5–3.5(–3.8) μm, L = 4 μm, W = 3.3 μm, Q = 1.2 (n = 30/1).

Type of rot: White rot.

Specimens examined: **CHINA. Yunnan Province**: Pu'er County, Laiyanghe Forest Park, on fallen angiosperm trunk, 6 June 2011, Dai 12234 (BJFC010517). **ECUADOR. Orellana Province**, Yasuni National Park, 9 March 2002, Ryvarden 44669 (O).

Comments: The Chinese material conforms closely to the Ecuadorian specimen and the original description, which cites slightly narrower basidiospores (3.5–4.5 × 2.5–3 μm; Ryvarden 1985).

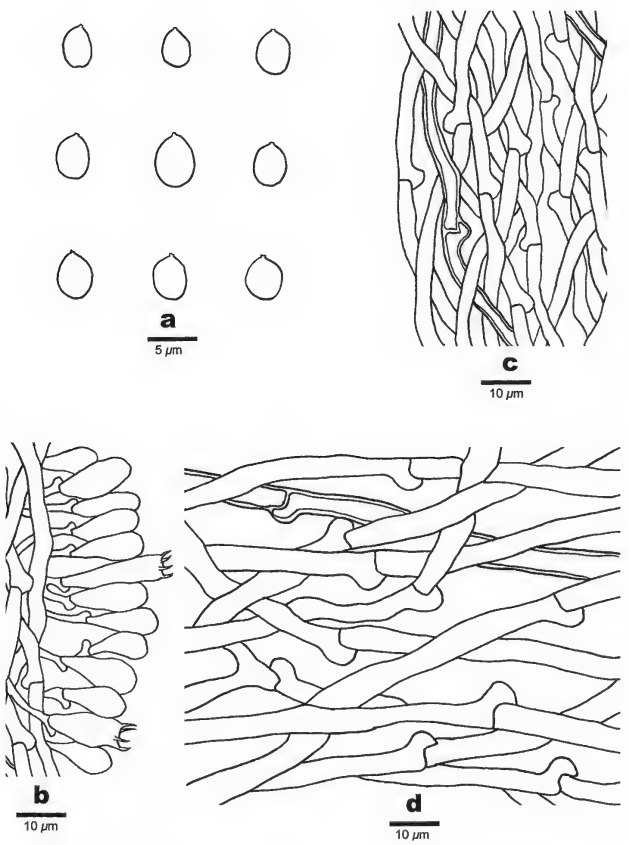

Fig. 5. *Tyromyces xuchilensis* (drawn from Dai 12234).
a. Basidiospores; b. Tramal section; c. Tramal hyphae; d. Context hyphae.

Discussion

The ITS+nLSU sequence analyses revealed seven major clades of *Polyporales*: antrodia clade, core polyporoid clade, fragiliporia clade, gelatoporia clade, phlebioid clade, residual polyporoid clade, and tyromyces clade (Fig. 1). The phylogenetic placement of the sequences from the three new Chinese records

was consistent with previous studies (Miettinen & Rajchenberg 2012; Binder et al. 2013).

In the phylogenetic tree, *Ceriporiopsis lagerheimii* was closely related to *C. guidella* Bernicchia & Ryvarden; however, *C. guidella* produces a dull yellow to green pore surface and has larger pores (4–5 per mm) and basidiospores (4–5 × 2.1–2.4 µm; Bernicchia & Ryvarden 2003; Ryvarden & Melo 2014). *Ceriporiopsis flavilutea* (Murrill) Ryvarden is similar to *C. lagerheimii* in having resupinate basidiocarps with white to cream pore surface, but it has larger pores (3–5 per mm) and ellipsoid basidiospores (3.5–4.5 × 2–2.5 µm; Ryvarden 1985). *Ceriporiopsis gilvescens* (Bres.) Domański resembles *C. lagerheimii* by having similar basidiospores (3.5–4.5 × 1.5–2 µm); however, *C. gilvescens* has an orange-brown pore surface, larger pores (4–5 per mm), and hyphae frequently covered by small, rod-like crystals (Ryvarden & Melo 2014). *Ceriporiopsis lagerheimii* was described from its type locality in Ecuador (Læssøe & Ryvarden 2010). The Chinese specimen (Dai 12304) clustered with the isotype (O, Ryvarden 58240) with strong support. This is the first report of *C. lagerheimii* from China.

Two *Sebipora aquosa* specimens from China (Dai 13592, Dai 13268) clustered with the holotype (ANDA, Miettinen 8868) and two other samples from Indonesia with strong support to form a sister lineage with *Gelatoporia subvermispora* (Pilát) Niemelä within the gelatoporia clade. Morphologically, *G. subvermispora* differs from *S. aquosa* by larger pores (2–4 per mm) and smaller allantoid basidiospores (4.5–5.5 × 1–1.5 µm; Miettinen & Rajchenberg 2012; Ryvarden & Melo 2014). This is the first report of *S. aquosa* from China.

Two *Tyromyces xuchilensis* specimens (Dai 12234 from China; Ryvarden 44669 from Ecuador) formed a strongly supported lineage not in the tyromyces clade but in the residual polyporoid clade. This unexpected result was also noted by Binder et al. (2013). The Mexican type specimen of *T. xuchilensis* (Murrill 1171) should be reexamined in order to resolve its taxonomic status. In the tree, *T. xuchilensis* appeared close to *Climacocystis borealis* (Fr.) Kotl. & Pouzar. However, *C. borealis* is easily distinguished from *T. xuchilensis* by its duplex context, larger pores (1–2 per mm), acute cystidia, larger basidiospores (4.5–6.5 × 3–4.5 µm), and habit on gymnosperms (Song et al. 2014). *Tyromyces xuchilensis* was described from Mexico, and later found in Ecuador (Ryvarden 1985); this is the first report of the species from China.

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Circinella (*Mucorales, Mucoromycotina*) from China

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Abstract—Nine taxa representing the genus *Circinella*—*C. angarensis*, *C. minor* var. *minor*, *C. minor* var. *asperior*, *C. mucoroides*, *C. muscae*, *C. nodulosa* sp. nov., *C. ramosa* sp. nov., *C. simplex*, *C. umbellata*—were recorded and described or redescribed from twenty-four different localities in seventeen cities of fifteen provinces in China. A key is provided to the nine taxa recorded from China. No zygosporic state was formed between any pair of the isolates studied.

Key words—*Mucoraceae,* morphology, new species, taxonomy

Introduction

Circinella (van Tieghem & Le Monnier 1873) is one of the smaller genera of the family *Mucoraceae* (*Mucorales*, *Mucoromycotina*). It is closely related to *Mucor* but differs in forming sporangiophores with circinate branches bearing sporangia with persistent walls rather than the non-circinate branches and non-persistent sporangial walls characteristic of *Mucor* species (Hesseltine & Fennell, 1955).

Hesseltine & Fennell (1955) recognized eight species in *Circinella.* Although subsequently seven additional species were included (Faurel & Schotter 1965; Mil'ko 1968, 1969, 1974; Patil & Kale 1981; Arambarri & Cabello 1996), six have since been accepted as representing other genera such as *Fennellomyces*, *Gongronella*, *Lentamyces*, *Mucor*, and *Pirella* (Benny & Benjamin 1975, Benny & Schipper 1992, Walther et al. 2013).

Six *Circinella* species have been described in Taiwan: *C. minor*, *C. mucoroides*, *C. muscae*, *C. rigida* [° *Mucor durus*], *C. simplex*, and *C. umbellata* (Yang & Liu 1972, Xiao et al. 2013).

In Mainland China, R.Y. Zheng isolated and identified *Circinella angarensis*, *C. minor*, *C. muscae*, and *C. umbellata*, which had been listed (but not described or illustrated) in Teng (1964) and Tai (1979). We collected and identified additional *Circinella* strains, adding four taxa (including two new species) to the Chinese records.

Of the nine *Circinella* taxa found in China described or redescribed in detail and illustrated here, eight have been recorded from Mainland China and one only from Taiwan.

Materials & methods

Isolation

All strains found in China were isolated from soils, dung, or rotten materials. The Warcup (1950) dilution plate method was used for soil samples, a small part of each dung sample was soaked in sterilized distilled water for 5–6 hours before using different dilutions to pour plates, and rotten materials were placed directly on the media.

Cultures

The isolates of each taxon studied are listed after the description of that taxon. Our research group isolated and identified the strains from Mainland China, and Prof. Hsiao-Man Ho supplied those from Taiwan. Dr. K. O'Donnell kindly supplied cultures from NRRL for comparative studies; Dr. A. Nakagiri kindly supplied culture NBRC 4453, sent by Dr. H. Naganishi. Living cultures are maintained in the Culture Collection of the State Key Laboratory of Mycology (accession numbers prefixed with 'C') and the China General Microbiological Culture Collection Center (CGMCC), and dried cultures are deposited in the Herbarium Mycologicum Academiae Sinicae (HMAS), all of the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

Media & cultivation

SMA (modified synthetic *Mucor* agar, containing dextrose 20 g, asparagines 2 g, KH_2PO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, thiamine chloride 0.5 mg, agar 20 g in 1000 mL distilled water, pH 7) was used for morphological studies. Color codes cited in the descriptions follow to Ridgway (1912). PDA, also adjusted to pH 7, was used for isolation, establishing temperature–growth relationships, and mating experiments. Cultivation period and temperature were 4–7 days at 18°C for isolating, 4–14 days at 25°C for morphological studies, 4 days at temperatures of 32–43°C for determination of the maximum growth temperature, and 7–20 days at 18°C for mating experiments.

Results & discussion

Maximum growth temperature

The 45 living cultures studied were tested twice for their maximum growth temperature. The eight species have a very similar maximum growth range: (31–)36–40°C, which may reflect the close affinity among these taxa. The number of strains is, however, too small to summarize the exact range for each

taxon. Since the results obtained are not distinctive enough to differentiate one another, the maximum growth temperature is not suitable for use in the identification of taxa in this study.

Mating experiments

Zygospores were not produced under different conditions between any of the mating pairs.

Morphological studies

As noted for many taxa in other mucoralean genera, morphological characteristics are found to be useful taxonomic criteria in distinguishing taxa within *Circinella*.

Taxonomy

Circinella Tiegh. & G. Le Monn., Ann. Sci. Nat., Bot., 5e sér., 17: 298. 1873.

Colonies fast growing, filling the Petri dish in 7–9 days at ±25°C, (3–) 7–10 mm high, at first white, then blackish in one week due to formation of sporangia; reverse occasionally wrinkled; with or without an unpleasant odor. Sporangiophores circinate, often in groups and borne alternately at the both sides of the main stems of the sporangiophores. Sporangia borne at the tip of the circinate branches, many-spored. Columellae well developed, variously shaped, always with a well-defined collar. Sporangiospores numerous, hyaline, globose to ovoid, always single-celled. Chlamydospores absent. Zygosporic state not found. (According to Hesseltine & Fennell (1955), zygosporangia [as "zygospores"] are nearly smooth-walled and produced between more or less equal and unadorned suspensors on special zygophores.)

Key to *Circinella* taxa recorded from China

1. Sporangiospores >10 µm diameter ..... ***C. angarensis***
1. Sporangiospores <10 µm diameter 2
2. Sporangiospores angular and irregular in shape ..... ***C. simplex***
2. Sporangiospores neither angular nor irregular in shape 3
3. Sporangia resistant to breaking; old colonies usually blackish grey ..... *C. rigida* [= ***Mucor durus***]
3. Sporangia easy to break; old colonies usually brownish 4
4. Sterile spines present on the sporangia-forming branches 5
4. Sterile spines absent on the sporangia-forming branches 6
5. Apex of columellae smooth ..... ***C. minor* var. *minor***
5. Apex of columellae verrucose ..... ***C. minor* var. *asperior***
6. Umbels with 2–12 sporangia ..... ***C. umbellata***
6. Umbels with 4–5 sporangia 7

7. Sporangiophores slightly undulate ..... *C. mucoroides*
7. Sporangiophores not undulate 8
8. Not forming special structures at the base of spines ..... *C. muscae*
8. Forming special structures at the base of spines 9
9. Special structures nodulose, not branched ..... *C. nodulosa*
9. Special structures not nodulose, branched ..... *C. ramosa*

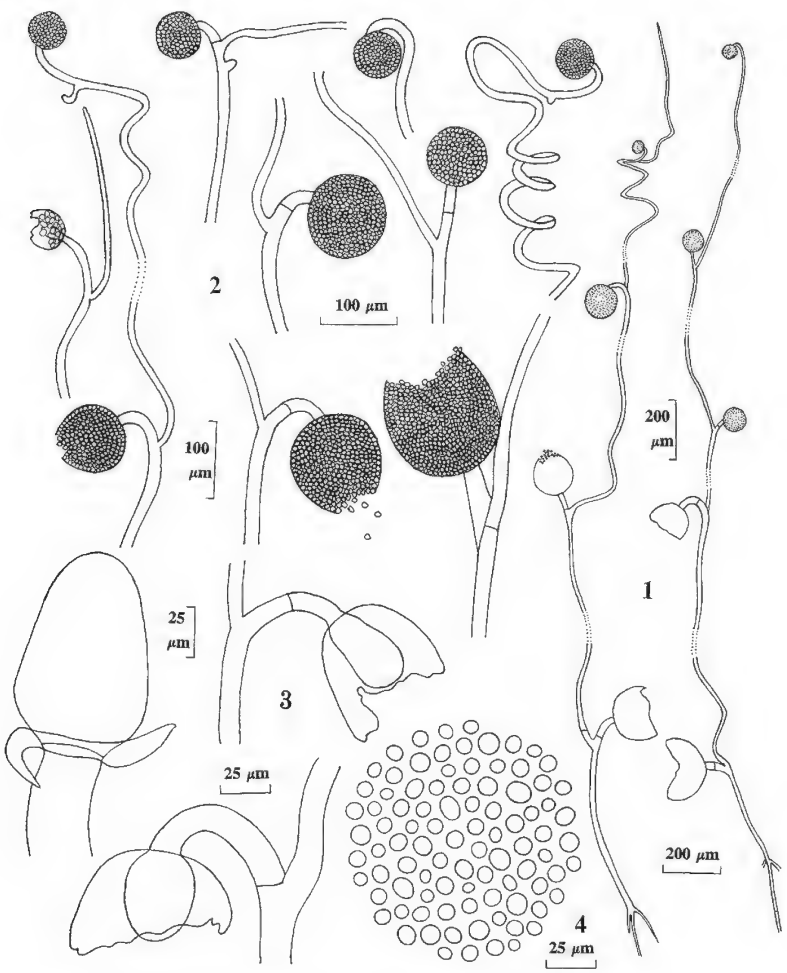

Fig. 1. *Circinella angarensis* (C43). 1. General characteristics of the sporophores; 2. Terminal portion of the sporangiophores; 3. Columellae with small or more often large pieces of the basal part of the sporangial wall attached;. 4. Sporangiospores.

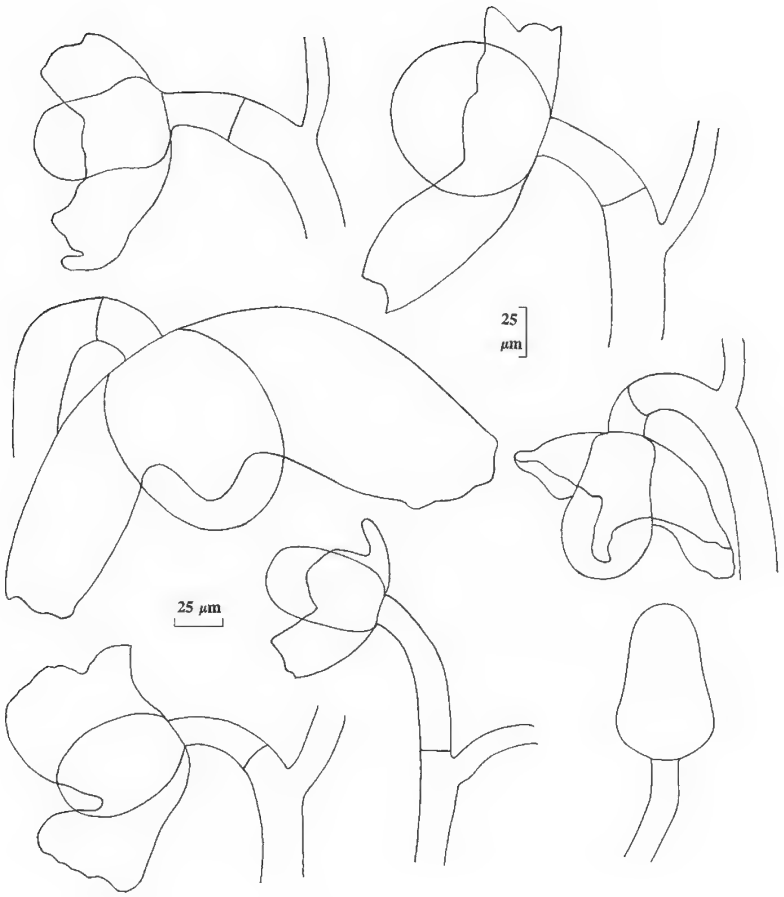

Fig. 2. *Circinella angarensis* (C43). More columellae with large pieces of sporangial wall attached.

Circinella angarensis (Schostak.) Zycha,
Krypt.-Fl. Brandenburg 6a: 98. 1935. Figs 1, 2
≡ *Mucor angarensis* Schostak., Ber. Deut. Bot. Ges. 15:473. 1897.

Colonies floccose, growing well at ±25°C, 6.8 cm in diameter and reaching 15 mm high on PDA, but 3.5 cm in diameter and 10 mm high on SMA. Odor none. At first white, then Light Drab to Cinnamon Drab or Buffy Brown, reverse Light Drab, margin irregular. Rhizoids present. Sporangiophores arising from both substrate and aerial mycelia, sub-straight to helicoid, main stems

11.5–24.5 μm diam., branches 6–10 μm diam., brownish colored, growing indefinitely and branching sympodially, branches with 1–2 sporangia, often accompanied by a spine. Lateral branches circinate, single or united into whorls and branching sympodially, branches with 1–2 sporangia, often accompanied by a spine. Sporangia (53–)83–145(–160) μm diam., at first hyaline, then blackish, with spores visible inside the sporangium, deliquescing after broken. Columellae ovoid and 61–82 × 44–60 μm, broadly cylindrical and 76 × 71 μm, cylindrical and 66–98.5 × 49–70 μm, nearly globose and 39–65 μm diam. Sporangiospores globose and 6–11 μm diam., or ovoid and 6.5–12 × 6–9.5 μm. Chlamydospores absent. Zygosporic state not formed between any pair of the (+) and (–) strains found in China under different conditions.

Strains studied: **China**, **Beijing**, Beijing Zoo, from dung of a special kind of pig, 30 April 1964, Zhe-fang Han (C43 = CGMCC 3.3396). **USA**, **California**, from rodent dung (C31 [= CBS 172.62 = NRRL 2628]); Los Angeles County, San Gabriel Mountains, from dung, R.K. Benjamin (C30 [=NRRL 2410, NT of *Mucor angarensis*]).

Circinella angarensis, originally described as *Mucor angarensis* by Schostakowitsch (1897), is recognized by having larger sporangia, columellae, and sporangiospores than other *Circinella* species. Zycha (1935) transferred this taxon to *Circinella* based on its original description. Hesseltine & Ellis (1961), who re-examined its isolates, confirmed this combination and proposed NRRL 2410 as neotype.

Circinella minor Lendn. var. ***minor***, Bull. Herb. Boissier, 2e sér., 5: 199. 1905. Fig. 3

Colonies floccose, growing well at ±25°C, 2–5(–10) mm or even higher; at first white, then Light Drab to Cinnamon Drab or Buffy Brown, reverse Light Drab Sporangiophores erect, main stems 8–18.5(–32) μm diam., branches 4–9(–14) μm diam. and 59–450 μm in length. Sporangia spherical, terminal ones (41–)57–82(–117) μm diam., lateral ones 29–70(–80) μm diam. Columellae broadly ovoid, pyriform or other shape, (27–)36–63(–99) × (20–)31–47(–68) μm, applanate or hemi-spherical, (7–)13.5–45 × (9–)16–63 μm. Sporangiospores spherical and 3.5–7(–9) μm diam., or ovoid and 4.5–7(–11) × 3.5–6.5(–9.5) μm,, hyaline. Chlamydospores absent. Zygosporic state not formed between any pair of the (+) and (–) strains found in China under different conditions.

Strains studied: **China**, **Shanxi**, Datong, from dung of black bear in zoo, 21 August 1963, Fu-mei Hu (C3 = CGMCC 3.14087); from horse dung, Fu-mei Hu (C16 = CGMCC 3.14100). **Beijing**, Zoological Institute, from cat dung, 3 August 1960, Hu Fu-mei (C8 = CGMCC 3.14092). **Hunan**, Hengyang City, Yueping Park, from soil, 17

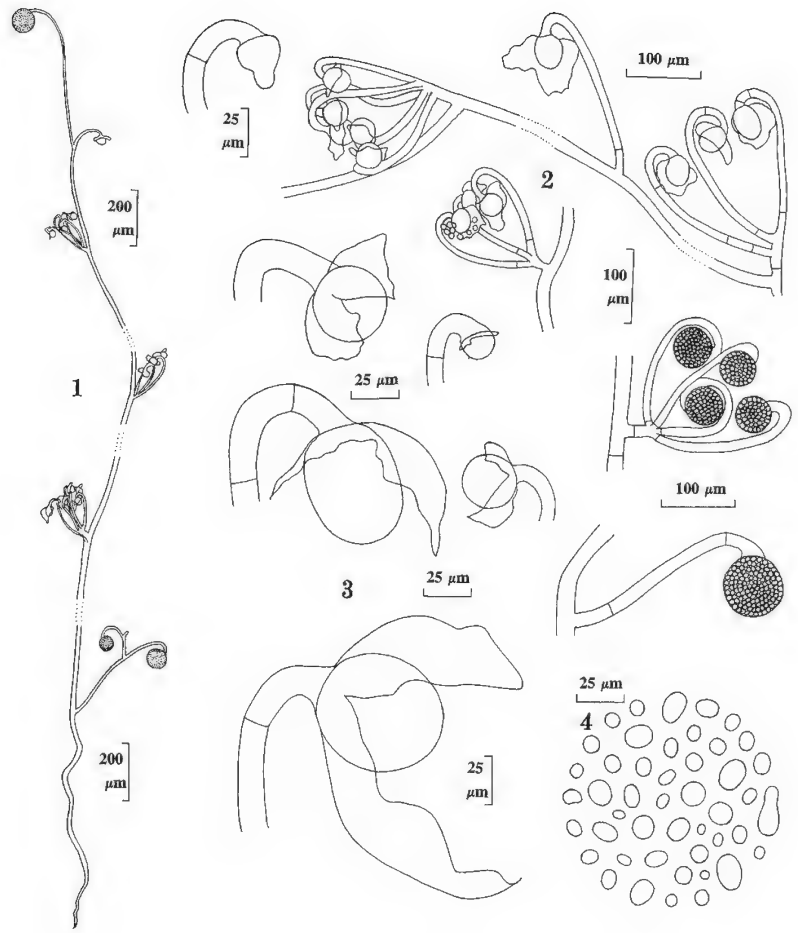

Fig. 3. *Circinella minor* var. *minor* (C3). 1. General characteristics of the sporophores; 2. Terminal portion of the sporangiophores; 3. Columellae often with large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores.

June 1963, Ru-yong Zheng & Fei-mei Hu (C11 = CGMCC 3.14095). **UNKNOWN**, A.F. Blakeslee (C32 [= NRRL 1353, = CBS 143.56]).

Circinella minor differs from other *Circinella* species in producing sporangiophores that terminate in an umbel of (1–)2–4(–6) sporangia and lacking a spine.

Circinella minor var. ***asperior*** M.O. Reinh.,
Ber. Deutsch. Bot. Ges. 45: 131. 1927. FIG. 4

COLONIES floccose, growing well at ±25°C, (2–)3–8(–10) mm high, at first white, becoming Old Gold at the 7th day, reverse milky white with pale brownish tint to Pale Orange Citrine, usually with disordered patches. Odorless, some nearly odorless. STOLONS and RHIZOIDS absent. SPORANGIOPHORES arising from the substrate or aerial mycelia, erect, growing indefinitely and

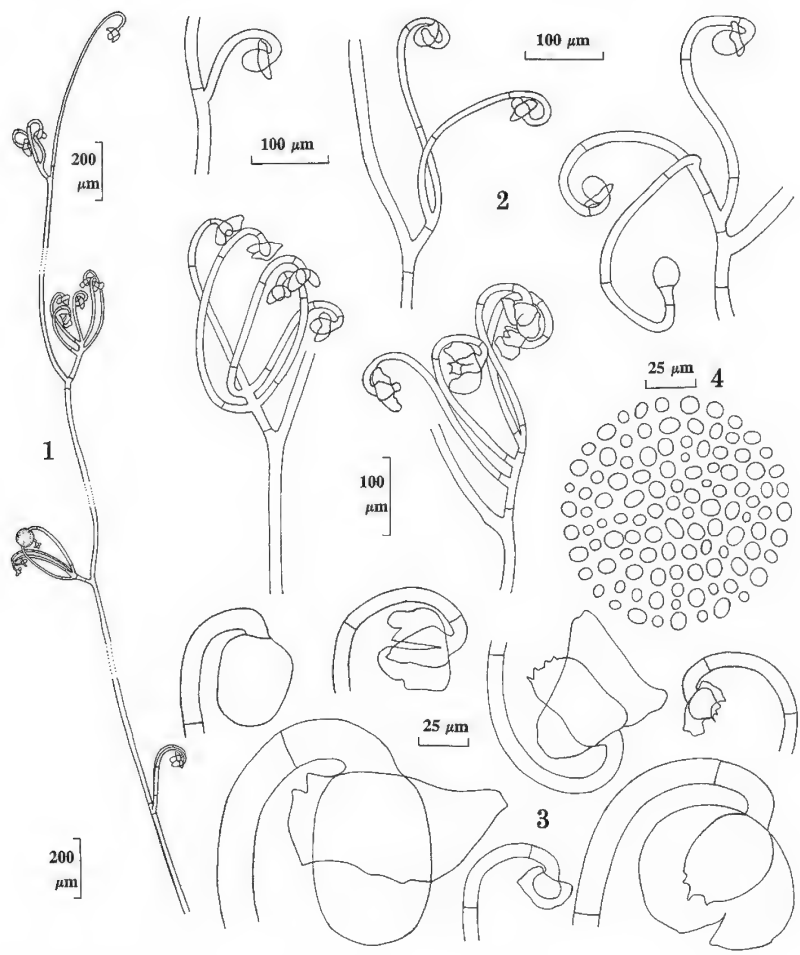

FIG. 4. *Circinella minor* var. *asperior* (C1). 1. General characteristics of the sporophores; 2. Terminal portions of the sporangiophores; 3. Columellae mostly with large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores.

branching sympodially, main stems (7–)11–23(–27.5) µm diam., branches (5.5–)7–9(–16) µm diam., (47–)82–200(–294) µm long, each branch with a single sporangium or more often terminating into an umbel of 2–5(–6) sporangia, hyaline to pale grayish-brown, 1–2(–4)-septate. Sporangia borne on circinate branches, spherical, both terminal and lateral sporangia similar in size, (32–)42–70(–100) µm diam., dark brown color, wall translucent, incrusted and broken. Columellae broadly ovoid or oblong-ovoid, (14–)27–70 × (15–)23–60 µm, smaller ones roundish conical, 23–46 × 23–55 µm, with 1 to several apical spines, brownish to grayish brown. Collar always present. Sporangiospores globose and 3.5–9(–10.5) µm diam., or ovoid and 5.5–10 × 4.5–9 µm. Chlamydospores absent. Zygosporic state not formed between any pair of the (+) and (–) strains found in China under different conditions.

Strains studied: **China**, **Hebei**, Tianjin City, People's Park, from rabbit dung, 27 May 1965, Zhe-fang Han (C1 = CGMCC 3.14085). **Guizhou**, Guiyang, from shrub forest soil, 8 June 1962, Fu-mei Hu (C5 = CGMCC 3.14089). **Ningxia**, Yinchuan, from pig dung, 19 October 1964, Fu-mei Hu (C17 = CGMCC 3.14101). **Beijing**, Mentougou, from mouse dung, 26 September 2002, Ji-qi Lu (C27 = CGMCC 3.14111). **Liaoning**, Dalian, December 2013 (C42 (= M.124).

Circinella minor var. *asperior* differs from var. *minor* by having many columellae with verrucae.

Circinella mucoroides Saito, Centralbl. Bakteriol., 2. Abth., 17: 159. 1907. Fig. 5

Colonies on SMA attaining 9 cm diam. in 8–9 days at 25°C, (2–)5–8(–10) mm high, at first white, becoming Dresden Brown or Buffy Citrine to Cinnamon Brown, Saccardo's Olive after 3 weeks, Antique Brown after 2 months. Reverse Buffy Citrine or Old Gold. Odorless in most strains, rarely with a moldy odor. Stolons and Rhizoids not seen. Sporangiophores arising from substrate or aerial mycelia, erect, growing indefinitely and branching sympodially from substrate or aerial mycelia, erect, growing indefinitely and branching sympodially, (70–)94–250(–505) µm in length, main stems (4.5–)9–18(–27.5) µm diam., branches (3.2–)6–11(–16) µm diam., each branch with 1(–2) sporangia, hyaline to pale brownish, 1–3-septate. Sporangia borne on circinate branches, spherical, terminal ones (21–)35–70(–82) µm, lateral ones similar in size, dark brown color, wall translucent and incrusted, breaking and leaving large pieces of basal collar. Columellae ovoid, cylindroid or near pyriform and 23–55(–69) × 16–46(–55) µm, globoid and (14.5–)41.5–55 µm, depressed globoid and 18.5–30 × 23–37 µm. Sporangiospores globose and 3.5–7(–9) µm diam, shortly ovoid and 5.0–7.5 × 4.5–6.5 µm. Chlamydospores not seen. Zygosporic state not formed between any pair of the (+) and (–) strains found in China under different conditions.

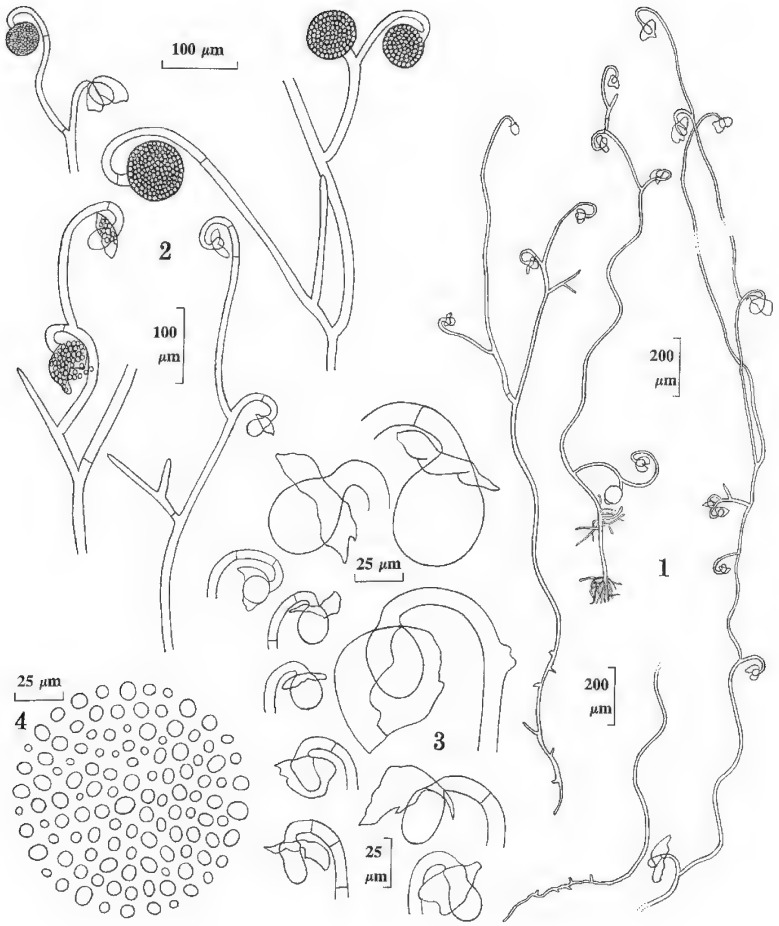

Fig. 5. *Circinella mucoroides* (C6). 1. General characteristics of the sporophores; 2.Terminal portion of the sporangiophores; 3. Columellae with large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores.

Strains studied: **CHINA**, **Guizhou**, Xiuwen County, from fallow soil, 21 November 1964, Qing-tao Chen (C2 = CGMCC 3.14086). **Jiangsu**, Nanjing Zoo, from fox dung, 12 October 1960, Xin-sheng Li & Fu-mei Hu (C6 = CGMCC 3.14090). **Zhejiang**, Hangzhou, Zhejiang Agricultural University, from swine dung, 4 September 1987, Gui-qing Chen & Fu-mei Hu (C19 = CGMCC 3.14103); from hen dung, Gui-qing Chen & Fu-mei Hu (C20 = CGMCC 3.14104). **Beijing**, Changping, from vegetable

garden soil, 4 May 1982, Ru-yong Zheng (C23 = CGMCC 3.14107); Mentougou, from mouse dung under a walnut tree, 26 September 2002, Ji-qi Lu (C28 = CGMCC 3.14112). **Yunnan**, Xishuangbanna, from soil under a tree, 9 July 1994, Gui-qing Chen (C26 = CGMCC 3.14110). **Inner Mongolia**, Taipushi, from mouse dung, Wei Liu (C29 = CGMCC 3.14113). **Taiwan**, 26 August 2014, Xiao-man He (C45, received as "*Circinella chinensis*"). **UNKNOWN**, A.F. Blakeslee (C33 [= NRRL 1354]).

Circinella mucoroides is diagnosed by its slightly undulate sporangiophores, simple sterile spines, and sporangia at the upper end of sporangiophores.

Circinella muscae (Sorokīn) Berl. & De Toni, Syll. Fung. 7: 216. 1888. Fig. 6
= *Circinella sydowii* Lendn., Bull. Bot. Genève, Ser. II, 5: 29. 1913.

Colonies on SMA floccose, attaining 9 cm diam. in 8–10 days at 25°C, 4–8(–10) mm high, at first white, becoming Buffy Citrine or Saccardo's Olive in 7 days, near Walnut Brown in 10 days and Antique Brown to Amber Brown after 2 months. Reverse Sayal Brown, with white to dirty yellow edge surrounded. Odor absent, rarely with a faint foul smell. Stolons absent. Rhizoids absent or present and branching profusely. Sporangiophores arising from substrate or aerial mycelia, erect, growing indefinitely and branching sympodially, (6.5–)9–14(–19) µm diam., branches (37–)74–447 µm in length, (4.5–)7–10 µm diam.; fertile branches circinate, with 1 or 2, sometimes 3 sporangia, mostly accompanied by a spine. Spines simple or dividing 1–2 times, stiff or circinate, recurved, or meandering, (47–)70–141(–376) µm long. Sporangia globose to slightly dorsiventrally flattened., 27–60(–82) µm diam., hyaline or translucent when young, dark gray to brown in age, wall persistent, breaking. Columellae variable in shape, larger ones pyriform, oblong-ovoid, constricted or not constricted at the middle portion, (14–)25–46 × (12–)19–32 µm, smaller ones conical or hemi-globose, (8–)12–30(–37) × (7–)11–25(–32) µm, mostly hyaline, rarely light brown, mostly smooth, a few with spines or protuberances at the apex, often with large pieces of collars attached. Sporangiospores globose and 3.5–7(–9) µm diam., ovoid and 4.5–7(–11) × 3.5–6.5(–9) µm. Zygosporic state not formed between any pair of the (+) and (–) strains found in China under different conditions.

Strains studied: **CHINA**, **Guangxi**, Guilin, from vegetable soil, Ru-yong Zheng (C13 = CGMCC 3.14097). **Hebei**, Kailuan, from underground cable, Zu-tong Qi (C21 = CGMCC 3.14105; C22 = CGMCC 3.14106; C24 = CGMCC 3.14108). **SOUTH AFRICA**, **Gauteng**, Johannesburg, from gold mine soil at a depth of 600 m and 40°C, A. Lendner (C41 [= NRRL 1364 = CBS 107.13, type of *Circinella sydowii*]). **UNKNOWN**, (C34 [= NRRL 1358]; C35 [= NRRL 1359]).

Circinella muscae is characterized by non-undulate sporangiophores and simple spines often associated with sporangiophore branches. Compared

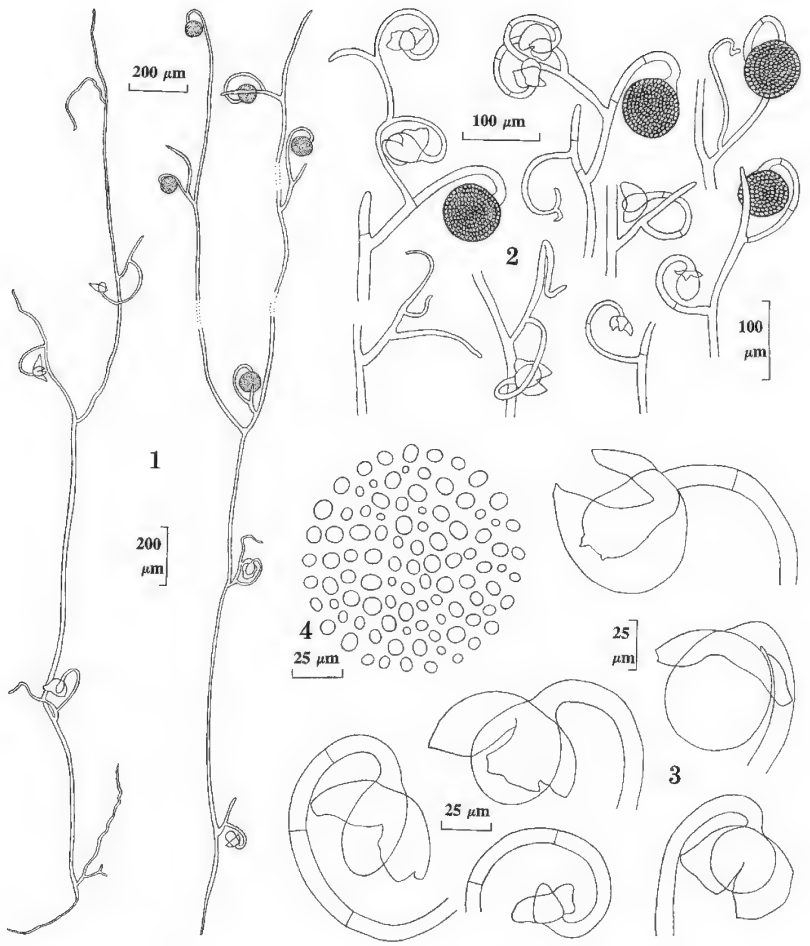

Fig. 6. *Circinella muscae* (C13). 1. General characteristics of the sporophores; 2.Terminal portion of the sporangiophores; 3. Columellae mostly with smooth apices and with large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores.

with its allied *C. mucoroides,* this species produces more deeply colored sporangiophores, fewer sterile spines, shorter and more circinate branches, and smaller sporangia.

Circinella nodulosa R.Y. Zheng, X.Y. Liu & Y.N. Wang, **sp. nov.** Fig. 7

Fungal Name FN570151

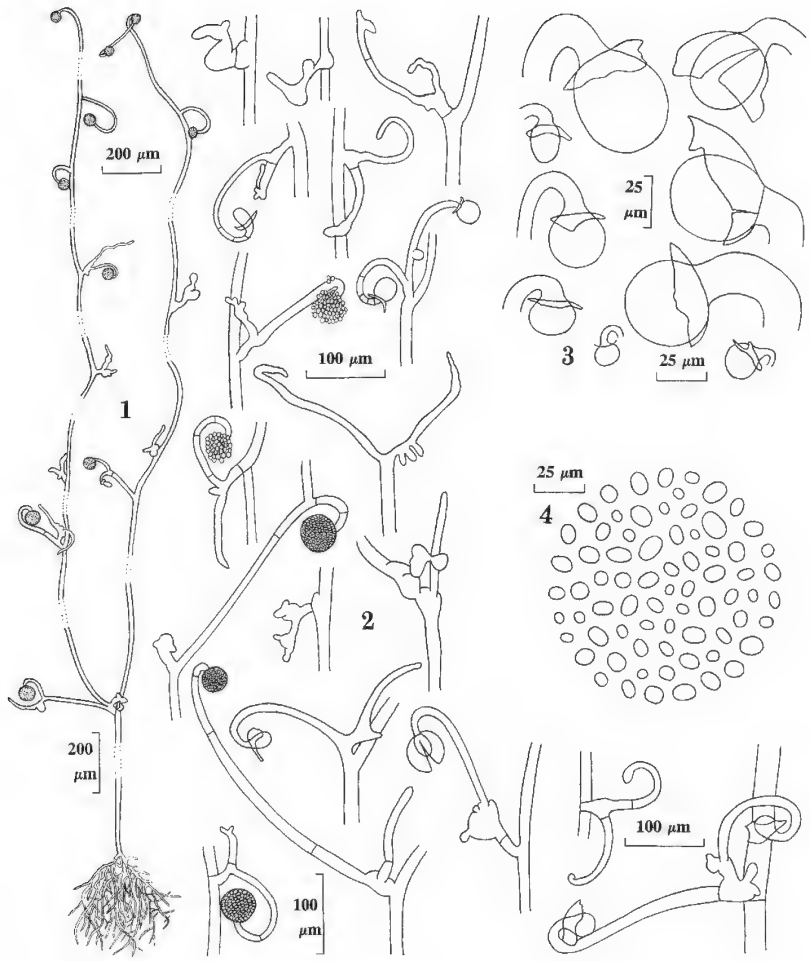

FIG. 7. *Circinella nodulosa* (C18). 1. General characteristics of the sporophores; 2. Terminal portion of the sporangiophores; 3. Columellae with small or large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores.

Differs from other species of *Circinella* by forming unbranched nodulose structures at the base of sporangiophores or spines.

TYPE: People's Republic of China, Zhejiang Prov., Hangzhou, campus of Zhejiang Agricultural University, from sheep dung, 4 September 1987, Gui-Qing Chen & Fu-Mei Hu (**Holotype** HMAS 245380; ex-holotype culture CGMCC 3.14102 = C18).

ETYMOLOGY: *nodulosa*, nodular, referring to the nodulose nature of the special structure forming at the base of spines.

Colonies on SMA floccose, attaining 9 cm diam. in 7–9 days at 25°C, about 10 mm high, at first white, becoming Yellow Ocher to Ochraceous Tawny in the central part, remaining white in the periphery. Reverse pale yellowish. Odorless. Stolons absent. Rhizoids abundant. Sporangiophores arising from the substrate or aerial mycelia, erect, growing indefinitely and branching sympodially, 59–450 µm in length; main stems 9–23(–50.5) µm diam., branches 4.5–11.5(–16) µm diam., each branch with 1(–2) sporangia, 1–3(–5)-septate on the branches, usually without septation on the main stems, nodulose outgrowths may appear on the main stems or the branches of the sporangiophores, especially at the base of the branches or spines. Sporangia borne on circinate branches, globose, 26–47 µm diam., light grayish brown to pale yellowish-brown or subhyaline, breaking and leaving a large collar. Columellae ovoid, 18.5–41.5(–50.5) × 16–32(–41.5) µm, globose to subglobose, 16–55 µm diam., smaller ones depressed-globose, 9–18.5 × 14–23 µm, pale to light brown. Collars small to large, usually without, but sometimes with apophyses. Sporangiospores ovoid and 5.5–9(–10) × 4–7.5(–8.5) µm, rarely reaching 12.5 × 9 µm, globose and (4.5–)5.5–7(–8) µm diam., hyaline. Zygosporic state not formed between any pair of the (+) and (–) strains found in China under different conditions.

Circinella ramosa R.Y. Zheng, X.Y. Liu & Y.N. Wang, **sp. nov.** Fig. 8

Fungal Name FN570150

Differs from other species of *Circinella* by sometimes forming, at the base of spines, a rhizoid-like structure that branches irregularly several times or is otherwise contorted.

Type: People's Republic of China, Inner Mongolia, from spinach field soil, June 1962, Xun-Chu Yan (**Holotype** HMAS 245379; ex-holotype culture CGMCC 3.14088 = C4).

Etymology: *ramosa*, from the Latin *ramosus* (branched), pertaining to the branched special structure forming at the base of spines.

Colonies on SMA attaining 9 cm in 7–9 days at 25°C, (2–)5–10 mm high, at first white, becoming Dark Citrine after 7 days in the central portion, or Saccardo's Olive after 20 days, with a white periphery which disappearing after 3 weeks. Reverse among Aniline Yellow and Orange Citrine in 7 days, 2–5 mm high. Near Odorless. Stolons absent. Rhizoids abundant and branching profusely at the base of the main stem. Sporangiophores arising from the substrate or aerial mycelia, erect, growing indefinitely and branching sympodially, curved to helicoids, (82–)141–400(–610) µm in length, main stems (8–)10–20.5(–25) µm diam., branches (4.5–)7–11.5(–13) µm diam., each branch with 1(–3) sporangia, or without sporangia and substituted by a

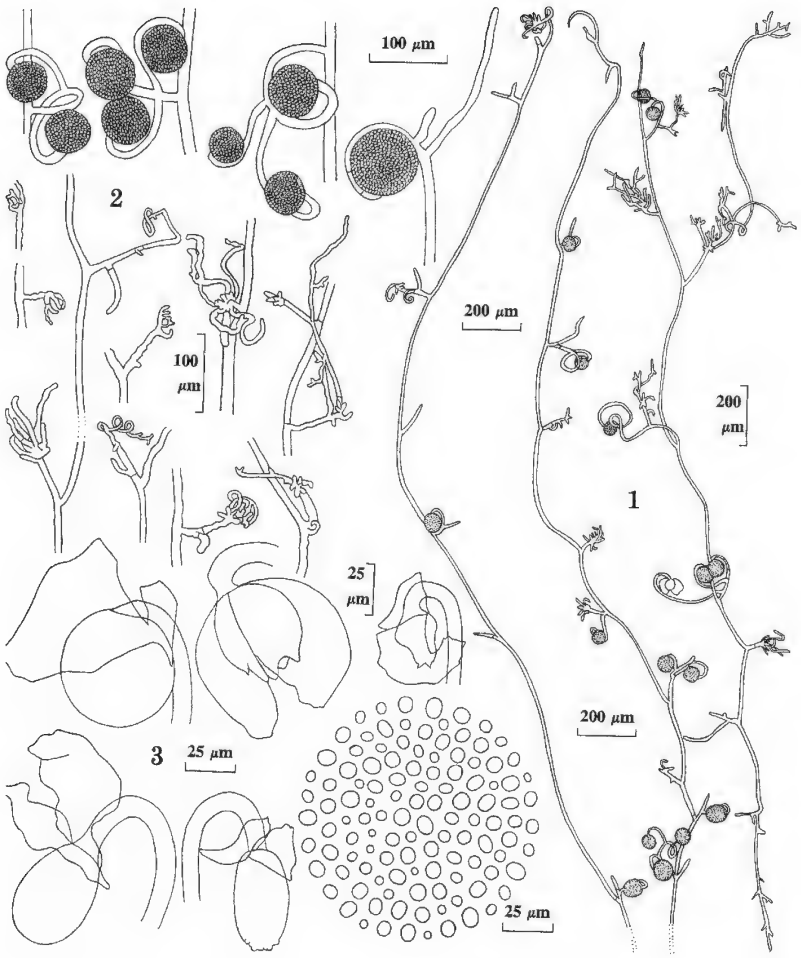

Fig. 8. *Circinella ramosa* (C4). 1. General characteristics of the sporophores; 2. Terminal portion of the sporangiophores; 3. Columellae with large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores.

rhizoid-like structure that branches irregularly several times or is contorted. Sporangia subhyaline, brownish to dark brown, somewhat depressed globose or spherical, (19–)33–75(–82) μm diam., broken after mature, collar large or small. Columellae ovoid, cylindroid, rarely pyriform, (18.5–)28–64(–74)

× (13–)30–48(–69) μm, or globose and (18.5–)27.5–46(–55) μm diam., upper most portion of the sporangiophores just beneath the sporangia often widened and apophyses-like. Sporangiospores ovoid and 4.5–9 × 3.5–8 μm, or globose and 3.5–7 μm diam., pale yellowish gray when single, gray color in mass. Zygosporic state not formed between any pair of the (+) and (–) strains found in China under different conditions.

Additional strains studied: **China**, **Guangdong**, Yingde, from soil under grasses, 4 October 1960, Ru-yong Zheng (C7 = CGMCC 3.14091). **Yunnan**, Yuanjiang, from dog dung, 21 May 1963, Zao-yuan Zhang & Yong-gui Ma (C10 = CGMCC 3.14094). **Hainan**, Chuangjiang, Cha River, from soil at river bottom, Dong-hai Ye (C14 = CGMCC 3.14098).

Circinella simplex Tiegh., Ann. Sci. Nat., Bot., 6e sér., 1: 92 (1875). Fig. 9

Colonies on SMA attaining 6.7 cm in 6 days at 25°C, at first white, 5–8 mm high, then submerged in the central portion, with a brownish zone which disappear after 3 weeks, Deep Olive-Buff to Dark Olive-Buff after 20 days, 2–3 mm high. Reverse Pale Olive-Buff. Near odorless. Stolons absent. Rhizoids absent. Sporangiophores arising from the substrate, erect, hyaline to pale brownish, growing indefinitely and branching sympodially, curved, (5–)8–12.5(–16) μm diam., branches slightly widening near the basal parts, (13.5–)31–68(–114) μm in length, each branch with one sporangium, branches (0–)1(–2)-septate, spines absent. Sporangia spherical, (27.5–)32–61.5(–69) μm diam., at first hyaline, then brown, deliquescing after broken. Collar small or absent. Columellae variable in shape, broadly ovoid, cylindroid or near pyriform, constricted or not constricted at the middle portion and (11–)14.5–37.5 (–44.5) × (7–)12.5–27.5(–41) μm, depressed globoid to conical and (5.5–)12.5–23 (–33) × (7–)14–25(–41) μm, mostly hyaline, the larger ones light brown, mostly smooth, a few with 1 to several spines at the apex. Sporangiospores very irregular in shape, mostly 3.5–9 × 2–7 μm, larger ones 10–16.5 × 4–5 μm diam., with conspicuous oil droplets, hyaline. Chlamydospores nearly spherical, 16–29 μm diam., each containing one larger or several small droplets, elongated on substrate mycelia. Zygosporic state not formed.

Strains studied: **China**, **Taiwan**, Taibei, from soil (C44 [= BCRC FU30085]). **Brazil**, from soil (C46 [= BCRC 31711 = CBS 142.35 = IFO 6412 = IMI 252490 = NRRL 2407]).

Circinella simplex has the typical morphological characteristics of this genus, circinate branches bearing sporangia with persistent walls, and was recognized by producing irregular sporangiospores.

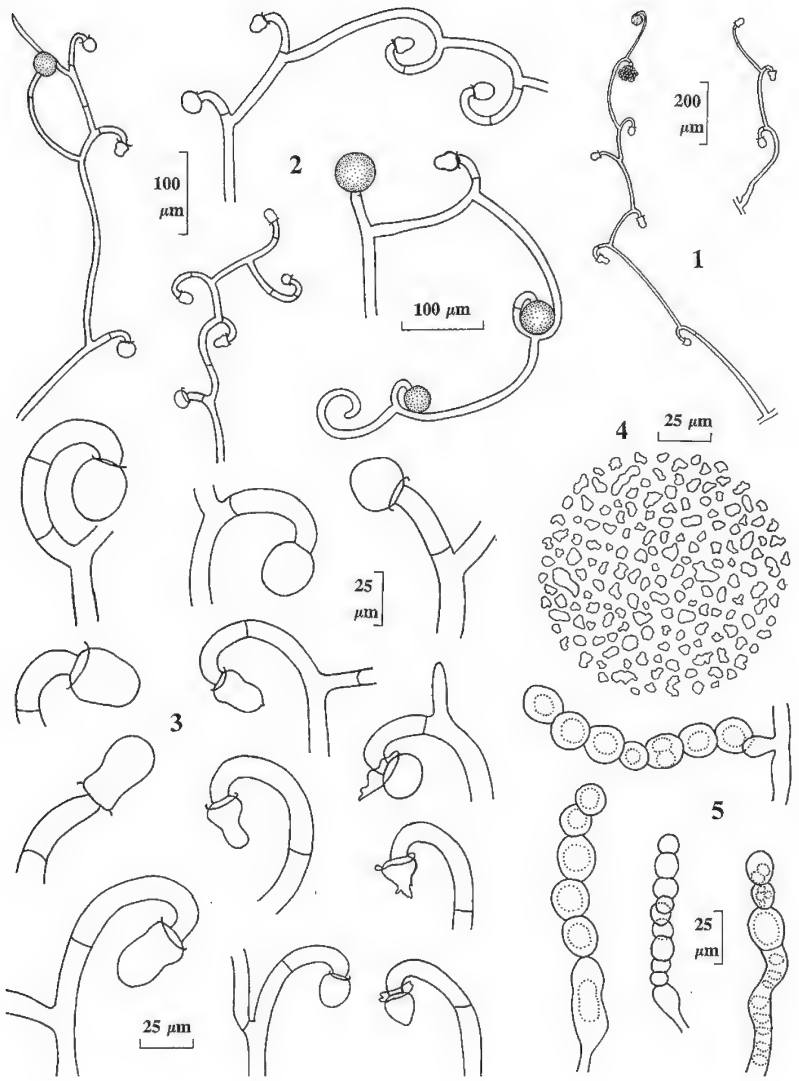

Fig. 9. *Circinella simplex* (C44). 1. General characteristics of the sporophores; 2. Terminal portion of the sporangiophores; 3. Columellae with small or large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores; 5. Chlamydospores.

Circinella umbellata Tiegh. & G. Le Monn.,
Ann. Sci. Nat., 5e sér., 17: 300. 1873. FIG. 10
= "*Circinella chinensis*" H. Nagan. &Kojiro, J. Ferm. Tech. 20: 409. Pl. 3. 1942, nom. inval.

COLONIES on SMA attaining 9 cm diam. in 7–9 days at 25°C, (3–)7–10 mm high, at first white, Old Gold or Isabella Color in 7 days, Light Brownish Olive to Cinnamon Brown in 2 weeks, then Hazel to Antique Brown after 2 months. Reverse similar to the obverse side or dirty grayish brown. STOLONS and RHIZOIDS absent. SPORANGIOPHORES arising from the substrate or aerial mycelia, erect, growing indefinitely and branching sympodially, (82–)130–258(–282) µm in length, main stems (9–)14–27.5(–33) µm diam., branches (5.5–)7–14(–18.5) µm diam., each branch with a single sporangium or more often terminating into an umbel of 2–12 SPORANGIA borne on circinate branches which are hyaline to somewhat brownish and (0–)1–2-septate, spherical, (27–)41–94(–118) µm diam., dark brown color, wall translucent, incrusted and breaking. COLUMELLAE ovoid, oblong-cylindroid, or pyriform and (23–)30–60(–74) × (18.5–)24–46(–64.5) µm, or globose and (18.5–)27.5–50.5(–69) µm diam. in the larger ones, depressed-globose to conical and (9–)18.5–41.5(–69) × (14–)23–46(–59) µm in the smaller ones, mostly smooth, a few with 1 to several spines at the apex, brownish or light grayish-brown. SPORANGIOSPORES globose, (4.5–)5.5–10 µm diam., or ovoid, (6–)7–11(–12.5) × (5.5–)6.5–10(–11) µm, easy to germinate and becoming irregular in shape. ZYGOSPORIC STATE not formed.

STRAINS STUDIED: **CHINA**, **BEIJING**, Zhongguancun, from mouse dung, 8 June 1961, Fu-mei Hu (C9 = CGMCC 14093). **HUNAN**, Hengyang, Yueping Park, from soil, 17 June 1963, Ru-yong Zheng & Fu-mei Hu (C12 = CGMCC 3.14096). **INNER MONGOLIA**, Arshaan, Yiershi, from wilted *Rhododendron* flower, 23 June 1963, Xi-ling Chen (C15 = CGMCC 3.14099). **HAINAN**, Limu Mountain, from soil under jackfruit plant, Song Lin (C25 = CGMCC 3.14109). **JAPAN** (C40 [= NBRC 4453 = CBS 140.28, type of "*Circinella chinensis*"]). **UNKNOWN**, A.F. Blakeslee (C36 [= NRRL 1351 = CBS 144.56]; from dog dung (C37 [= NRRL 1366 = CBS 145.56]).

Circinella umbellata, the type species for *Circinella,* differs from other species in the genus in its sporangiophores that terminate in an umbel of 2–12 sporangia. *Circinella "chinensis",* which was published by Naganishi & Kojiro (1942) without a Latin diagnosis, was treated by Hesseltine & Fennell (1955) as a synonym of *C. mucoroides.* Naganishi (1974), however, pointed out sporangiophore differences by comparing the Osaka strain (IFO 4455) of *C. mucoroides* and Dairen strain (NRRL 1354) of *C. chinensis.* Our study of the ex-type of *C. "chinensis"* reveals that morphologically it belongs to *C. umbellata.*

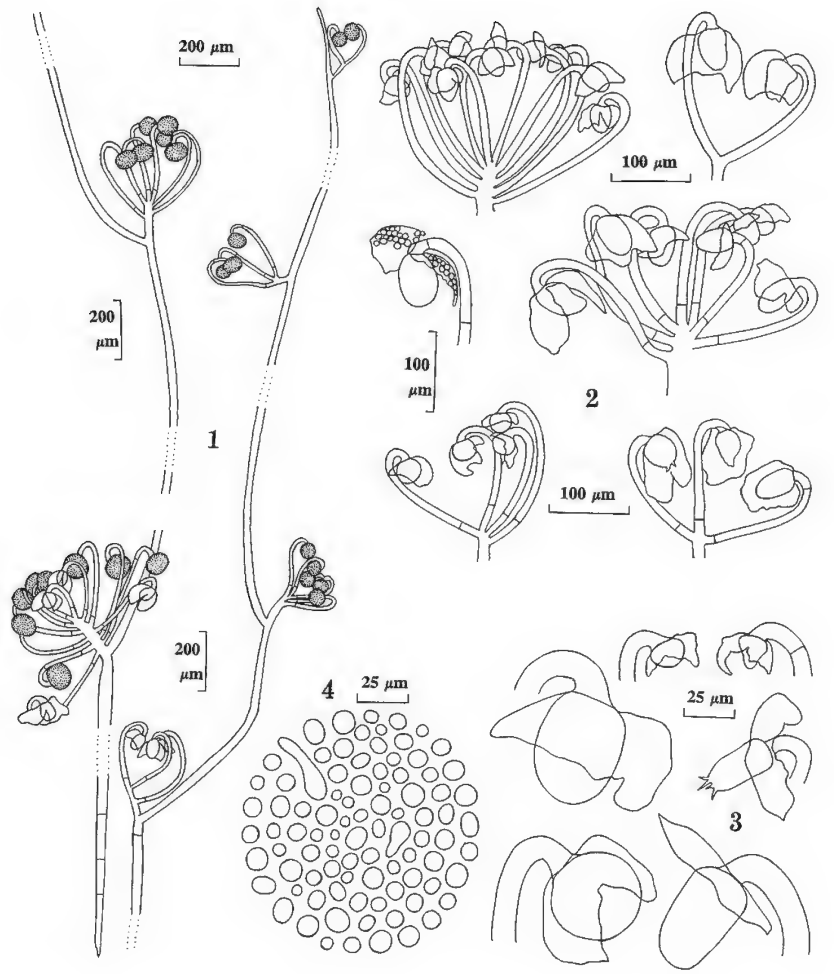

FIG. 10. *Circinella umbellata* (C9). 1. General characteristics of the sporophores; 2.Terminal portion of the sporangiophores; 3. Columellae usually with large pieces of the basal part of the sporangial wall attached; 4. Sporangiospores.

Acknowledgments

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Astraeus ryoocheoninii sp. nov. from Korea and Japan and phylogenetic relationships within *Astraeus*

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Abstract—The morphological characteristics of a collection by C.I. Ryoo and K.-H. Ka from the demilitarized zone (DMZ) of Korea, invalidly published as "*Astraeus koreana*", are re-evaluated, described, and illustrated. Phylogenetic analysis using nrITS sequence data indicate that "*Astraeus koreana*", an unnamed "Japanese *Astraeus* group 2", and a Japanese specimen E0059827 all differ from *A. hygrometricus* var. *koreanus* and represent a new species, proposed here as *Astraeus ryoocheoninii*.

Key words—basidiospore, ornamentations, peridium, ectomycorrhiza

Introduction

Species of *Astraeus* are ectomycorrhizal and form symbiotic associations with various tree species in the genera *Alnus*, *Castanea*, *Eucalyptus*, *Pinus*, and *Pseudotsuga* (Malajczuk et al. 1982, Molina 1979, Nouhra & Domínguez 1998, Trappe 1967). Mycorrhizal synthesis studies have been conducted to confirm their host specificity in the field (Danielson 1984; Petcharat 2005; Molina 1979, 1981; Molina & Trappe 1982). Mycorrhizae carry out the efficient recycling of nutrients between roots of host plant and fungi in soil (Malajczuk et al. 1982). *Astraeus* species are also economically important as they are collected for sale in commercial markets as edible mushrooms (Maiti et al. 2008).

Astraeus (*Sclerodermatineae, Boletales*, *Agaricomycetes*) was originally described by Morgan (1889). The genus contains seven widely distributed species (http://www.mycobank.org) and has been recorded from Australasia, Africa, Asia, Europe, and North and South America (Coker & Couch 1928, Cunningham 1944, Dring 1964, Imazeki & Hongo 1989, Liu 1984, Nouhra & Domínguez 1998, Wilson et al. 2012). The type species, *A. hygrometricus* (Pers.) Morgan, was originally described in *Geastrum*; a Korean variety has been described as *A. hygrometricus* var. *koreanus* V.J. Staněk [≡ *A. koreanus* (V.J. Staněk) Kreisel] (Staněk 1958, Kreisel 1976). Several new *Astraeus* species have been described recently from Asia: *A. asiaticus* Phosri et al., *A. odoratus* Phosri et al. [= *A. thailandicus* Petcharat], and *A. sirindhorniae* Watling et al. (Petcharat 2005; Phosri et al. 2004, 2007, 2014). Recently *Astraeus hygrometricus* var. *koreanus* and an unnamed *Astraeus* taxon from Japan ("Clade VII, Japanese *Astraeus* group 2" of Fangfuk et al. 2010) were morphologically and molecularly re-evaluated (Fangfuk et al. 2010). However, their relationship was left unresolved, and Fangfuk et al. (2010) concluded that further studies were necessary to determine the phylogenetic position of var. *koreanus* and the identification of "Japanese *Astraeus* group 2."

Until 2001, *Astraeus* was largely defined by two species, *A. hygrometricus* and *A. pteridis* (Kirk et al. 2001, Zeller 1948). Since then several new species have been described using morphological characteristics and molecular methods (Phosri et al. 2004, 2007; Fangfuk et al. 2010). *Astraeus asiaticus* and *A. odoratus* were described as phylogenetically distinct from *A. pteridis*. SEM was used to characterize basidiospore ornamentation as a character in a taxonomic key of the genus. Phosri et al. (2007) suggested that the *A. hygrometricus* complex forms a polyphyletic group in ITS rDNA sequence analyses. These studies showed that in *Astraeus* phylogenetic clades largely correlate with geographical areas. Fangfuk et al. (2010) also confirmed polyphyletic relationships within *Astraeus*.

From 1995 to 2000, researchers from NIFoS (National Institute of Forest Science) conducted an ecological investigation in the Korean demilitarized zone (DMZ) including the civilian control zone (CCZ). In 1998, an undetermined *Astraeus* species was collected from the DMZ, described, and photographed by C.I. Ryoo and K.-H. Ka. This collection was named "*A. koreana*" by Ryoo et al. (1999), but the name was invalid because no Latin description was included and no type specimen was designated. "*Astraeus koreana*" is macro-morphologically similar to *A. hygrometricus* var. *koreanus* (Staněk 1958) and shares micro-morphological characteristics with "Japanese *Astraeus* group 2" (Fangfuk et al. 2010).

Our study taxonomically re-evaluated *A. hygrometricus* var. *koreanus*, "*A. koreana*", and "Japanese *Astraeus* group 2" by using phylogenetic analysis to

identify which morphological characteristics are important for distinguishing taxonomic boundaries. *Astraeus ryoocheoninii* is proposed as a new species incorporating "*Astraeus koreana*" and "Japanese *Astraeus* group 2".

Materials & methods

Morphological characteristics

We studied the macro-morphological characteristics of fresh material as recorded and photographed in 1998 by C.I. Ryoo & K.-H. Ka and their dried material conserved in the herbarium of National Institute of Forest Science, Hongnung Arboretum, Seoul, the Republic of Korea (KFI). Micro-morphological features were described from dried materials mounted in 3% KOH, Melzer's reagent, and cotton blue reagent under a Leica DM 2500 microscope using DIC optics. Basidiospore ornamentation was examined under a Hitachi S-3500N electron microscope using samples coated in platinum palladium at 10 kV.

Molecular analysis

DNA was extracted from dried material using the CTAB (cetyltrimethyl ammonium bromide) buffer method of Rogers & Bendich (1994). The rDNA ITS region was amplified by PCR using primers ITS1 and ITS4 (White et al. 1990). A 50 µl total volume per PCR reaction contained template DNA (1.2 µl), 10× buffer, 0.5 M KCl, 0.1 M Tris-HCl, 0.1 % Triton X-100, and 15 mM $MgCl_2$, 2.5 mM dNTP, 100 pM of each primer, and Taq polymerase (5 unit/µl). PCR thermal cycler protocols comprised 35 cycles of 30 sec at 94 °C, 30 sec at 56 °C, and 1 min at 72 °C with the first denaturation and last extension times extended to 5 min at 72 °C (Gardes & Bruns 1993). Cycle sequencing was performed using the primers ITS1 and ITS4 using BigDye™ cycle sequencing kit, version 3.1 (Applied Biosystems). Purified DNAs were directly sequenced on an ABI Prism TM 377 DNA automatic DNA Sequencer (Applied Biosystems).

Phylogenetic analysis

A total of 28 sequences, one newly extracted in this study and 27 retrieved from GenBank, were aligned using Clustal X (Thompson et al. 1997) implemented in the program MEGA v 5.05 (Tamura et al. 2011). Parsimony analyses were performed in PAUP* v 4.0b10 (Swofford 2002) with bootstrap statistics generated using a heuristic search with 1000 replicates, each with 10 random sequence additions, and TBR branch swapping. Bayesian analyses were performed in MrBayes v 3.1 (Ronquist & Huelsenbeck 2003) using 2,000,000 generations and 4 chains under the general time reversible (GTR) model with gamma-distributed substitution rates. Trees were saved every 100th generation, and the first 1000 of 2001 trees were discarded as the burn-in. A 50% majority rule consensus tree on treefiles from Bayesian and parsimony bootstrap analyses was used to compute posterior probabilities (PPs) and bootstrapping (BS) values respectively. The outgroup of *Scleroderma verrucosum* (Genbank AJ629886) and *Pisolithus* sp. (AJ629887) was used in the systematic dataset following the methods by Phosri et al. (2007). Sequences used in this study are listed in Table 1.

TABLE 1. List of *Astraeus*, *Pisolithus*, and *Scleroderma* sequences used in the phylogenetic analysis

TAXON	COUNTRY	HOST PLANT	GENBANK NO.
A. asiaticus	Thailand	—	AJ629381
	Thailand	—	AJ629382
	Thailand	—	AJ629386
A. hygrometricus	USA	—	AJ629398
	USA	—	AJ629399
	USA	—	AJ629402
	USA	—	AJ629403
	Greece	—	AJ629404
	Spain	—	AJ629408
A. hygrometricus var. *koreanus*	Japan	*Pinus thunbergii*	AB535105
	Japan	*Pinus thunbergii*	AB535106
	Japan	*Pinus thunbergii*	AB535107
A. odoratus	Thailand	*Dipterocarpaceae*	AB507406
	Thailand	—	AB535108
	Thailand	*Dipterocarpaceae*	AB535113
	Thailand	*Dipterocarpaceae*	AJ629875
	Thailand	—	AJ629880
A. pteridis	USA	—	AJ629409
	USA	—	AJ629410
A. ryoocheoninii	**Korea**	***Pinus densiflora***	**KC985146**
	Japan	—	AB507397
	Japan	—	AB535109
	Japan	*Pinus densiflora*	AB535110
	Japan	*Quercus serrata*	AB535111
	Japan	*Quercus* sp.	AB535112
	Japan	*Carpinus laxiflora*	AJ629405
Pisolithus sp.	Thailand	—	AJ629887
S. verrucosum	Spain	—	AJ629886

Results

Phylogenetic analysis

Based on nrITS sequence data obtained in this study and from GenBank, phylogenetic placement and relationships of *Astraeus* taxa were inferred from the Bayesian and MP analyses. No difference was found between the topologies using different methodologies so only the results from the Bayesian analysis is shown (FIG. 1). The aligned dataset included 595 total characters, with 393 constant, 85 variable, and 117 parsimony informative in MP analysis. This dataset produced 1,000 trees with 315 steps of tree length, a consistency

index (CI) of 0.8349, a homoplasy index (HI) of 0.1651, and a retention index (RI) of 0.9091.

Five *Astraeus* clades are recognized in Bayesian and parsimony analyses (Fig. 1). Clade A, representing *A. hygrometricus*, is subdivided into three geographical subclades: North American (0.81 PP, 0.76% BS); South European (0.92 PP, 91% BS); and Japanese (0.80 PP, 83% BS); the Japanese subclade represents *A. hygrometricus* var. *koreana*, which is confirmed as a variety rather than a species as suggested by Kreisel (1976). Clade B, here named *A. ryoocheoninii*, includes "*A. koreana*", "Japanese *Astraeus* group 2",

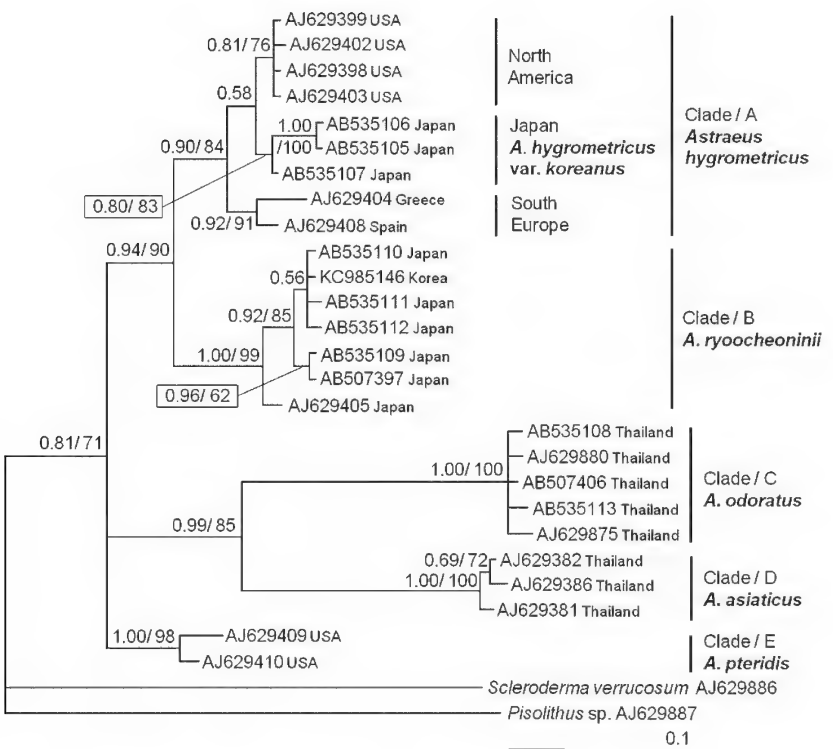

Fig. 1. Phylogenetic analysis for the genus *Astraeus* based on (ITS1+5.8S+ITS2) rDNA region. Bayesian consensus tree used to a 50% majority-rule carried out two million generations and four chains. Maximum parsimony analysis generated from 1000 replicates with 10 random addition sequences. Because no differences were found between the tree topologies from the two analyses, only the MCMC tree is shown. Numbers at each branch indicate posterior probability (left) and bootstrap support (right).

and Japanese specimen E00159827 (GenBank AJ629405) in a monophyletic clade (1.00 PP, 99% BS). The other three monophyletic clades represent: clade C—*A. odoratus* (1.00 PP, 100% BS); clade D—*A. asiaticus* (1.00 PP, 100% BS); and clade E—*A. pteridis* (1.00 PP, 98% BS).

Taxonomy

Astraeus ryoocheoninii Ryoo **sp. nov.** FIG. 2

MYCOBANK MB 804156

"*Astraeus koreana*" C.I. Ryoo, KMS Newsletter 11(2): 20, 1999, nom. inval.

"Japanese *Astraeus* group 2" Fangfuk et al., Mycoscience 51: 291–299, 2010.

Differs from *Astraeus hygrometricus* var. *koreanus* by its larger basidiomes and smaller basidiospores.

TYPE—The Republic of Korea, Central and Eastern mountainous region near the DMZ of Korea, Kosung, Mt. Hangrohbong, alt. ± 850 m, on sandy soil associated with *Pinus densiflora*, 20 July 1998, C.I. Ryoo & K.-H. Ka (**Holotype**, KFI-DMZ002 ; GenBank KC985146).

ETYMOLOGY—Named in honour of mycologist, Dr. Cheon-In Ryoo.

BASIDIOME globose, rough, with an ostiole encircled by a cracked peridium 60–100 mm wide when mature; the exoperidium splitting longitudinally and re-curving into star-like rays, expanding when exposed to moisture and re-coiling towards gleba upon desiccation. EXOPERIDIUM slightly viscous, smooth, grayish brown, covering endoperidium, splitting into 15–19(–21) little, star-like rays that open when hydrated, upright at first, curving back, inner surface becoming distinctly cracked with irregular trapezoid and rhomboid patterns, closing in dry weather. ENDOPERIDIUM a thin membranous layer of tissue, enclosing the spore mass in a 20–40 mm diameter sac, nearly round, with an irregularly shaped ostiole at top, surface felty rough, whitish, becoming gray to brown. BASIDIOSPORE mass whitish when young, brownish and powdery at maturity. BASIDIOSPORES globose, (5.3–)7.0–9.1(–9.5) µm in diameter (n = 48), (4.8–)5.0–9.0 µm in diameter without the ornamentation, chocolate dark brown, cell wall thickened, with spiny, moderately dense ornamentation with rounded (sometime spiked) top, (0.5–)0.6–1.3(–1.5) µm long, dark brown, negative reaction from 3% KOH, Melzer's reagent (inamyloid) and cotton blue reagent. PARACAPILLITIUM hyphae (4.0–)5.0–6.0(–6.5) µm in diameter, septate, with clamp-connection-like structures present on some septa.

ORIGINAL DESCRIPTION OF "*ASTRAEUS KOREANA*" (Ryoo et al. 1999): "These specimens were characterized by having the inner surface of the exoperidium distinctly cracked in irregular patterns, numerous, 15–19(–21) rays and 5.7–9.3 µm average size of basidiospores with short, moderately dense ornamentation, growing symbiotic in association with *Pinus densiflora*. Korean common name of these taxa were called as Seonbi-meonji-beoseos."

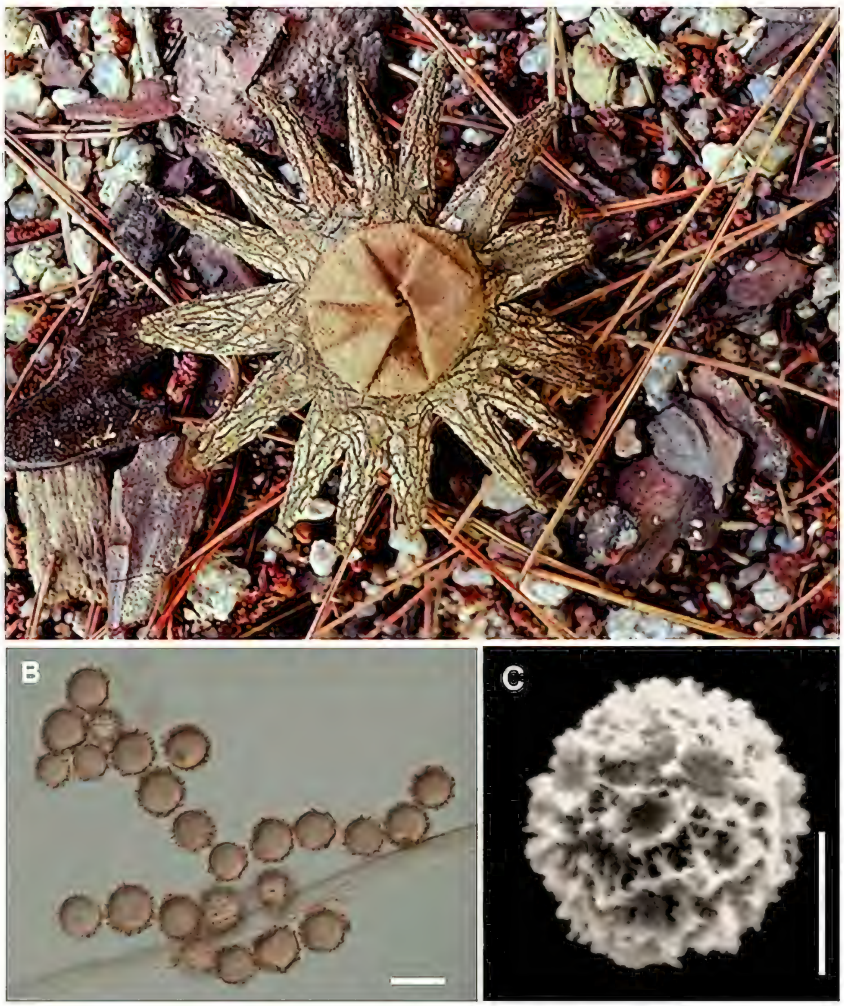

Fig. 2. *Astraeus ryoocheoninii* (KFI-DMZ002, holotype). A. basidiomata; B. basidiospores under DIC microscope; C. basidiospores under SEM. Scale bars: B = 10 µm; C = 5 µm

Additional specimens—Japanese specimens now included in *A. ryoocheoninii* were listed and described in Phosri et al. (2007, as ASTRAE_94 = E00159827, GenBank AJ629405), and in Fangfuk et al. (2010, as "Japanese *Astraeus* group 2").

Comments — The Japanese specimens of *Astraeus ryoocheoninii* agree with the Korean holotype description in basidiospore size and dense ornamentation with short spines (Table 2).

Table 2. Comparison of taxonomic characteristics of *Astraeus hygrometricus* and Asian *Astraeus* species

Taxon	Acute rays	Spore size (µm)	Spore ornamentation	Clade
A. hygrometricus	6–15[a]	5.2–13.9[a]	Dense, narrow, 0.7–1.3 µm[b]	A
A. hygrometricus var. *koreanus*	15–22[a]	7.6–12.9[a]	Tightly dense, narrow, 0.8–1.6 µm[a]	A
A. ryoocheoninii – "*A. koreana*"	15–21	5.3–9.5	Slightly dense, 0.6–1.3 µm	B
A. ryoocheoninii – "*Astraeus* group 2"	5–13[a]	5.2–9.6[a]	Moderately dense, 0.6–1.7 µm[a]	B
A. ryoocheoninii – AJ629405	—	6.8–9.0[a]	Dense, 0.8–1.6 µm[b]	B
A. odoratus	3–9[c]	7.5–15.2[c]	Moderately dense, narrow, 1.0–1.7 µm[c]	C
A. asiaticus	5–12[b]	8.8–15.2[b]	Very dense, 0.9–1.45 µm[b]	D

[a] Fangfuk et al. (2010); [b] Phosri et al. (2007); [c] Phosri et al. (2004).

The *A. ryoocheoninii* holotype is similar to *A. hygrometricus* var. *koreanus* in the high number of split acute rays, but *A. hygrometricus* var. *koreanus* differs by its larger basidiospores with longer, denser ornamentation (Table 2), much smaller basidiocarps (15–20 mm), and much thinner endoperidium (10–15 mm; Staněk 1958; Imazeki & Hongo 1989; Fangfuk et al. 2010). The type locality of *A. ryoocheoninii* was a warm, dry locality associated with stands of Korean red pine (*Pinus densiflora*) on sandy soil, whereas the type locality of *A. hygrometricus* var. *koreanus* was a stony plateau with an eastern orientation among dwarf-oaks and scattered pines in the mountains of North Korea (Staněk 1958: 632, 819).

The two Thai species, *A. odoratus* and *A. asiaticus*, have larger basidiospores and fewer cracked rays than *A. ryoocheoninii* (Table 2). The basidiospore spines of *A. odoratus* are narrower, more coalescent, and less densely arranged than those of *A. ryoocheoninii* (Phosri et al. 2004, 2007; Table 2).

Discussion

The studies of Phosri et al. (2007) and Fangfuk et al. (2010) resolved several questions concerning the phylogenetic relationships of the *Astraeus* species. The present study uses ITS rDNA variation to establish the systematic relationship among *A. hygrometricus* var. *koreanus*, "*A. koreana*", and "Japanese *Astraeus* group 2", and to identify morphological characteristics

that can be used to identify these species. This study suggests that *Astraeus hygrometricus* var. *koreanus* should be recognized at the rank of variety within the *A. hygrometricus* complex, rather than at specific rank as proposed by Kreisel (1976). The new species *Astraeus ryoocheoninii* is described for the well-supported clade that includes the sequences of "*A. koreana*", "Japanese *Astraeus* group 2", and Japanese specimen E00159827 (GenBank AJ629405), previously loosely associated with *A. hygrometricus* by Phosri et al. 2007 and with "Japanese *Astraeus* group 1" by Fangfuk et al. 2010).

Acknowledgments

This paper is dedicated to the memory of Dr. Cheon-In Ryoo (1951–2009). The authors appreciate Vladimír Antonín (Moravian Museum, Czech Republic) for his comments about the nomenclature. We wish to thank our reviewers, Dr. Andrew W. Wilson (Purdue University, USA) for revising the manuscript and valuable comments and Dr. Mikael Jeppson (Sweden) and Dr. Ronald H. Petersen (University of Tennessee, USA) for critical remarks. This work was supported by project (FP 0801-2010-01) of National Institute of Forest Science.

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New records of *Caloplaca, Hydropunctaria,* and *Verrucaria* from Turkey and Asia

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Abstract—Three lichen species—*Caloplaca oleicola*, *Hydropunctaria adriatica*, and *Verrucaria elaeina*—are described as new to Turkey and to Asia.

Key words—*Ascomycota*, *Corylus* sp., lichenized fungi, *Teloschistaceae*, *Verrucariaceae*

Introduction

The lichen biota of Turkey has received considerable attention in recent years. However, additional and more intensive lichenological field studies are needed for a complete lichen checklist of Turkey, because some regions of Turkey have not been investigated. Additional lichen records for Turkey are reported almost every year (e.g. Oran & Öztürk 2010, Arslan et al. 2011, Karagöz et al. 2011, Kınalıoğlu & Aptroot 2011, Vondrák et al. 2012, Aslan & Yazıcı 2013, Çobanoğlu et al. 2013, Yazıcı & Aptroot 2015, Şenkardeşler et al. 2016), including species new to science (e.g., Aptroot & Yazıcı 2012). In this paper we describe three lichen species: *Caloplaca oleicola*, *Hydropunctaria adriatica*, and *Verrucaria elaeina*, as new records for Turkey and Asia.

Materials & methods

The specimens examined are deposited at the herbarium of the Biology Department, Faculty of Science and Arts, Giresun University, Giresun, Turkey (GUB). They were studied with standard anatomical and morphological methods with the help of traditional chemical reagents (K, C, P, and I) used in lichenology (Smith et al. 2009).

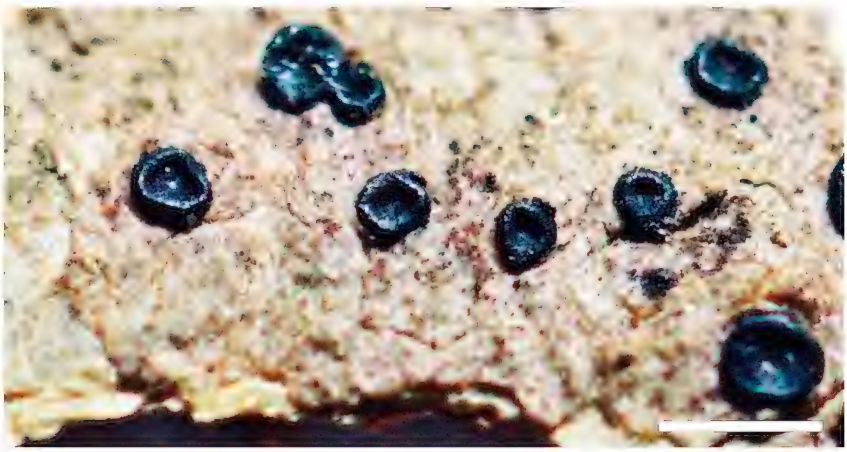

Fig. 1. *Caloplaca oleicola* (GUB 2387): habit. Scale bar = 1 mm.

Taxonomy

Caloplaca oleicola (J. Steiner) van den Boom & Breuss,
Mycotaxon 56: 131. 1995. Fig. 1

Thallus thin, white or pale grey, smooth to very slightly rimose. Apothecia black, 0.3–0.7 mm diam., usually scattered, without a thalline margin, proper margin persistent, well-delimited and mostly glossy, disc slightly concave to flat. Epihymenium dark brown. Ascospores colourless, 10–15 × 5.5–9 µm, ellipsoid, septum 3.5–5 µm wide. Thallus C–, K–, KC–, PD–; apothecial proper margin and epithecium C–, K+violet-red, KC–, PD–.

Specimen examined: **TURKEY. Giresun:** Piraziz, Aydere village, 40°56′33″N 38°06′46″E, 156 m, on *Corylus* sp., 30 March 2006, leg. K.Kınalıoğlu, det. A. Khodosovtsev (GUB 2387).

Remarks—Our Turkish collection had apothecia and ascospores of a similar size to those described in the *Caloplaca oleicola* protologue (apothecia <0.6 mm diam, ascospores 10–16 × 6–8 µm with 5 µm septum; Boom & Etayo 1995).

Caloplaca oleicola is an uncommon corticolous species with black apothecia, previously found only on bark of *Alnus incana* and *Olea europaea* in Europe and North America (Boom & Etayo 1995, Goward et al. 1996); but in Turkey, we collected it from smooth bark of *Corylus* sp. in a shady and humid hazelnut garden. This is the first report of the species from Asia.

Caloplaca oleicola is clearly distinguished from *C. servitiana* by its thin white thallus and lack of a thalline exciple (Vondrák et al. 2010).

FIG. 2. *Hydropunctaria adriatica* (GUB 2388): habit. Scale bar = 1 mm.

Hydropunctaria adriatica (Zahlbr.) C. Keller & Gueidan,
Taxon 58(1): 194. 2009. FIG. 2

Thallus epilithic, superficial, shiny black. Perithecia scarce, prominent, concolorous with thallus, globose, 0.3–0.5 mm in diam. Ascospores ovoid, oblong, 12.5–17.5 × 6–8.75 µm. Pycnidia not observed. Thallus and apothecia C–, K–, KC–, PD–.

SPECIMEN EXAMINED: **TURKEY. GIRESUN:** Keşap, Değirmenağzi village, sea shore (mostly intertidal zone), 40°58′20″N 38°37′23″E, 1 m, on siliceous rock, 11 April 2010, leg. K.Kınalıoğlu, det. A. Orange (GUB 2388).

REMARKS— Our Turkish sample is distinguished from the European samples by its substrate preference, its smaller apothecia, and somewhat bigger ascospores; European material had apothecia 0.45–1 mm in diam and ascospores 12–15 × 7.5–8.5 µm (Zschacke 1933–34).

This poorly known species was previously found on calcareous rocks splashed by seawater on the shores of the Adriatic, Aegean, Black, and Mediterranean Seas (Zschacke 1933–34, Rechinger 1951, Orange 2012). It is common in the Adriatic and Aegean Seas where it forms the so-called "black band" on seashore rocks (Rechinger 1951). In Turkey, it grows mostly on intertidal siliceous rocks on the Black Sea shoreline. This is the first report of the species from Asia.

Hydropunctaria adriatica differs from *H. maura* usually by its thinner thallus, lack of a dark basal layer, and exciple unpigmented below (Zschacke 1933–3), Orange 2012).

Verrucaria elaeina Borrer, in Smith & Sowerby, Engl. Bot. 35: t.2455 f.2. 1813. Fig. 3

Thallus epilithic, continuous, smooth, pale green-brown, rimose. Perithecia numerous, one-quarter to three-quarters immersed in the thallus, but depth varies in the same samples, forming low to moderate projections 0.2–0.4 µm in diam. Involucrellum well-developed, hemispherical to conical, dark reddish-brown. Ascospores ellipsoid-oblong or ellipsoid to narrowly ellipsoid, 15–20 × 6.5–8 µm. Conidiomata not observed. Thallus C–, K–, KC–, PD–.

Specimens examined: **TURKEY. Giresun:** Piraziz, Aydere village, 40°56′33″N 38°06′46″E, 156 m, on siliceous rock, 30 March 2006, leg. K.Kınalıoğlu, det. A. Orange (GUB 2389); Trabzon: Araklı, Konakönü place, sea shore, 40°57′17″N 40°02′56″E, 3 m, on siliceous rock, 12 August 2006, leg. K.Kınalıoğlu, det. A. Orange (GUB 2390).

Remarks— Our Turkish collections had perithecial projections of a similar size to those described by Orange (2000) and Smith et al. (2009), which had perithecial projections up to 0.22–0.4 mm. in diam. However, Turkish ascospores are smaller than those in previous descriptions (15–24 × 6–9.5 µm; Orange 2000, Smith et al. 2009). One Turkish specimen differs ecologically in occurring on siliceous rocks on the seashore.

Verrucaria elaeina is a common species occurring on a wide variety of substrata such as shaded sandstone, limestone, calcareous mudstone, concrete, siliceous rock and brick, in woodland or beneath herbaceous vegetation, on vast areas of ground in gardens, on damp walls, by unused railways and on ruined buildings, often on stones embedded in the ground, cliffs, weakly calcareous rock in shade and occasionally beside streams, also on paths, in the British Isles and Europe (Smith et al. 2009, Orange 2000). In Turkey, we collected it from siliceous rocks in moist shady hazelnut gardens and on the seashore. This is the first report of the species from Asia.

The pale grey-green thallus of *Verrucaria elaeina* on shaded rocks is characteristic and often easily recognized by the naked eye. Some poorly developed morphs have a much less distinctive appearance and could be

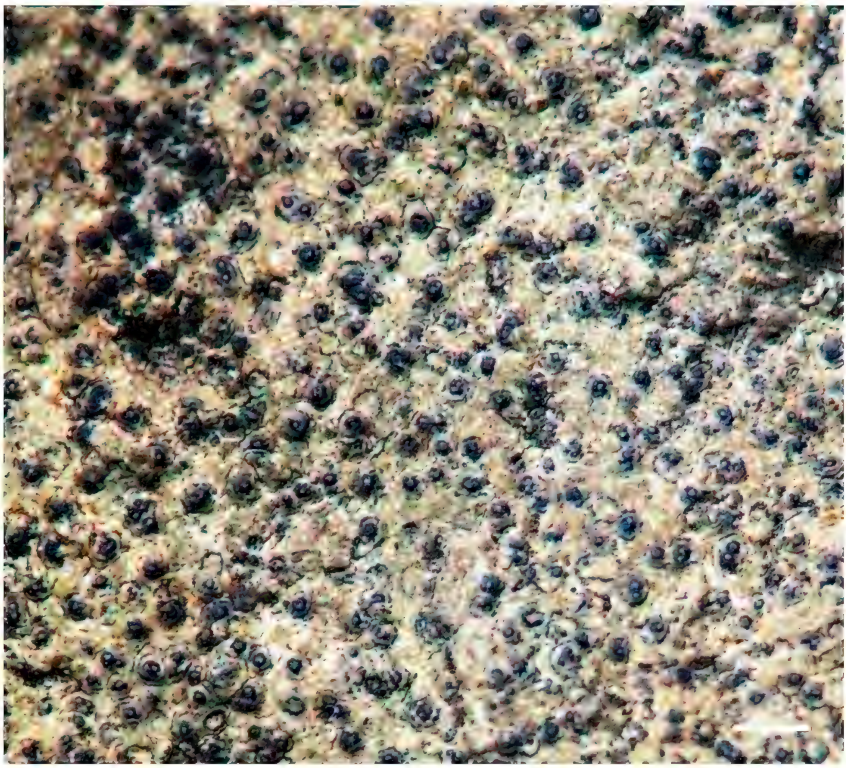

FIG. 3. *Verrucaria elaeina* (GUB 2389): habit. Scale bar = 1 mm.

confused with *V. denudata* or *V. dolosa*. *Verrucaria praetermissa* differs in its thicker thallus, more immersed perithecia, slightly larger ascospores, and its typically freshwater habitat (Smith et al. 2009).

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Chloridium terricola sp. nov. from China

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Abstract—A new species, *Chloridium terricola*, was isolated from soil in Sichuan province, China. Morphologically, it is characterized by the production of pale brown conidia and a conidiophore bearing a single apical whorl of ampulliform or lageniform phialides. Large subunit (LSU) rDNA sequence analyses support *C. terricola* as congeneric with other *Chloridium* species.

Key words—hyphomycetes, ITS, phylogenetic, soil fungi, systematics

Introduction

For competing sexually vs. asexually typified generic names in *Sordariomycetes* (except *Diaporthales*, *Hypocreales*, and *Magnaporthales*), Réblová et al. (2016) have recommended the name *Chloridium* Link 1809 based on its priority and widespread use, proposing *Gonytrichum* Nees & T. Nees 1818 and *Melanopsammella* Höhn. 1920 as generic synonyms. In 2005, four hyphomycete isolates morphologically similar to *Chloridium* were obtained from soil in the west region of Sichuan province, China. Their taxonomy was further evaluated through close microscopical examination and large subunit

(LSU) rDNA sequence analyses. These morphological and phylogenetic analyses revealed that the four isolates represented a unique undescribed *Chloridium* species, for which we propose the name *Chloridium terricola.*

Materials & methods

Fungal strains & morphology

Soil samples were collected in Sichuan Province, China, and transported to the laboratory in sterilized, zip lock polyethylene bags. All isolates are maintained in the Plant Pathology Herbarium, Guizhou University, Guiyang, China (HGUP). Mycelium grown from the soil samples was transferred to fresh 1.5% potato dextrose agar (PDA) medium plates. Cultural characteristics and morphology were determined on PDA and incubated for 7 d at 25°C under a 12 h/12 h light and darkness cycle, to promote sporulation. Water solution of 60% (v/v) lactic acid without a color dye was used as the mounting medium. Dimensions are based on 50 mature conidia and 30 conidiophores as measured at 100× magnification under a Nikon 90i microscope.

DNA amplification & sequencing

Extracted DNA was amplified via PCR using primers ITS4 and ITS5 for the ITS region and primers LROR and LR5 for the LSU region (Vilgalys & Hester, 1990; White et al. 1990). Additional sequences were obtained from GenBank. After the sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), the alignments were refined manually. Our DNA sequences were deposited in GenBank.

Phylogenetic analyses

The sequence data were subjected to parsimony analysis using the heuristic search option of PAUP* version 4.0b10 (Swofford 2002). MP (maximum parsimony) trees were inferred using heuristic search option with tree bisection reconnection (TBR) branch swapping and 1000 random sequence additions with maxtrees at 5000, branches of zero length collapsed, and all parsimonious trees saved. Measures calculated for parsimony included tree length (TL), consistency index (CI), retention index (RI), and rescaled consistence index (RC). Bootstrap analyses (Hillis & Bull 1993) were conducted with 1000 replications.

Taxonomy

Our morphological and molecular phylogenetic analyses support recognition of our four soil isolates as one phenotypically and phylogenetically distinct species.

Chloridium terricola Yong Wang bis, Jie & K.D. Hyde, **sp. nov.** Fig. 1

MycoBank MB 820926

Differs from *Chloridium gonytrichii* by its single whorl of phialides at the conidiophore apex and by its light brown conidia.

Type: China, Sichuan Province, Jiuzhaigou, isolated from soil, August 2005, Yu-Lan Jiang (**Holotype**, HGUPd4519 [dried culture]; ex-type culture, HGUP4519; GenBank KM434144, KT893302).

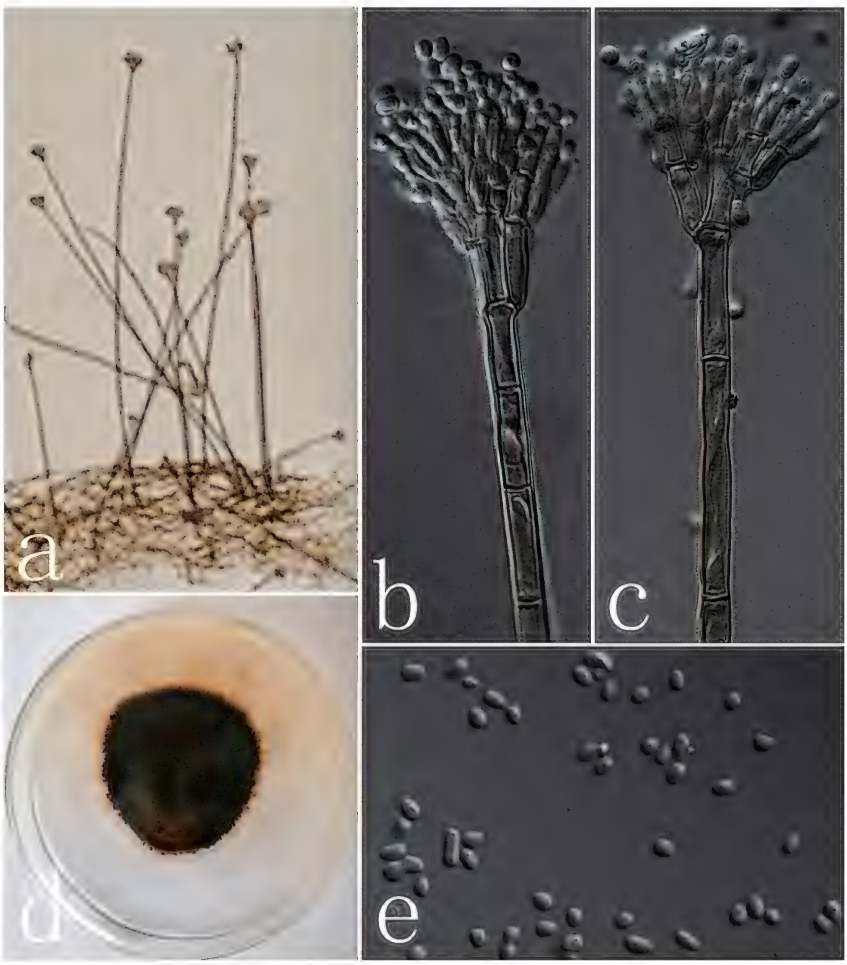


Fig. 1 (color). *Chloridium terricola* (HGUPd4519). a: Conidiophores arising from PDA. b-c: Conidiogenous cells, conidia, and conidiophores. d: Colony on PDA at 7d. e: Conidia. Scale bars: c-d = 20 μm; e = 10 μm.

Etymology: Latin, *terricola* meaning living on the soil.

Colonies effuse, velvety, reverse dark brown to black. Mycelium partly immersed, partly superficial, composed of branched, hyaline, septate, smooth, 3.5–5 μm wide hypha. Conidiophores mononematous, erect arising from the hypha, hyaline to pale brown, smooth. Conidiogenous cells borne directly on the hypha or in divergent forks on the conidiophores, ampulliform

or lageniform, pale brown, smooth, 7–15 × 2.5–4 µm. Conidia obovoid or ellipsoid, truncate at the base, pale brown, smooth, 2–3 × 2–2.5 µm. Sexual stage not observed.

Additional specimens examined: **China, Sichuan Province**: Panzhihua city, isolated from soil, August 2005, Yu-Lan Jiang (HGUP4509;GenBank KM434143, KT893301); Chengdu city, isolated from rice soil, August 2005, Yu-Lan Jiang (HGUP4536; GenBank KM434146, KT893304); Leshan city, isolated from soil, August 2005, Yu-Lan Jiang (HGUP4521; GenBank KM434145, KT893303).

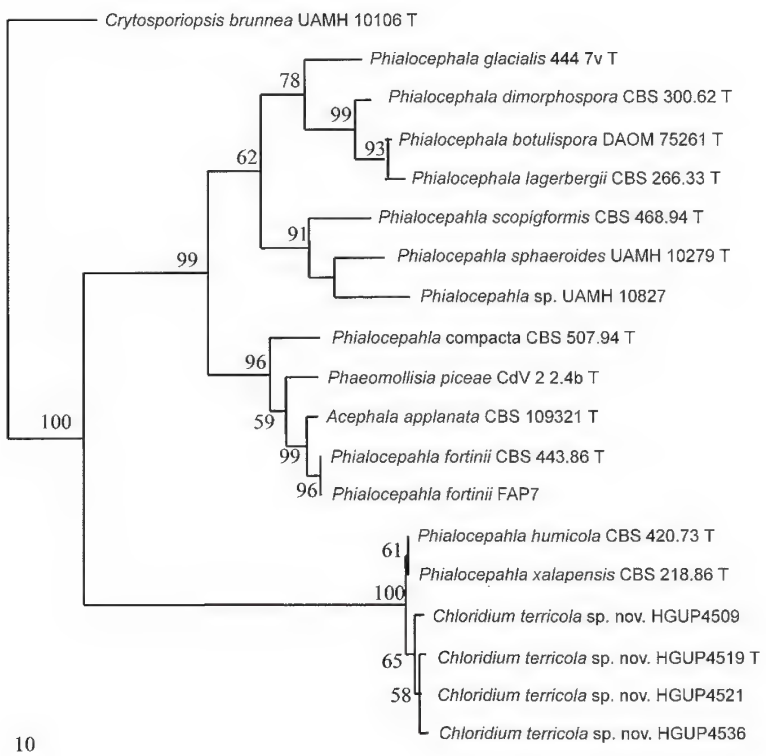
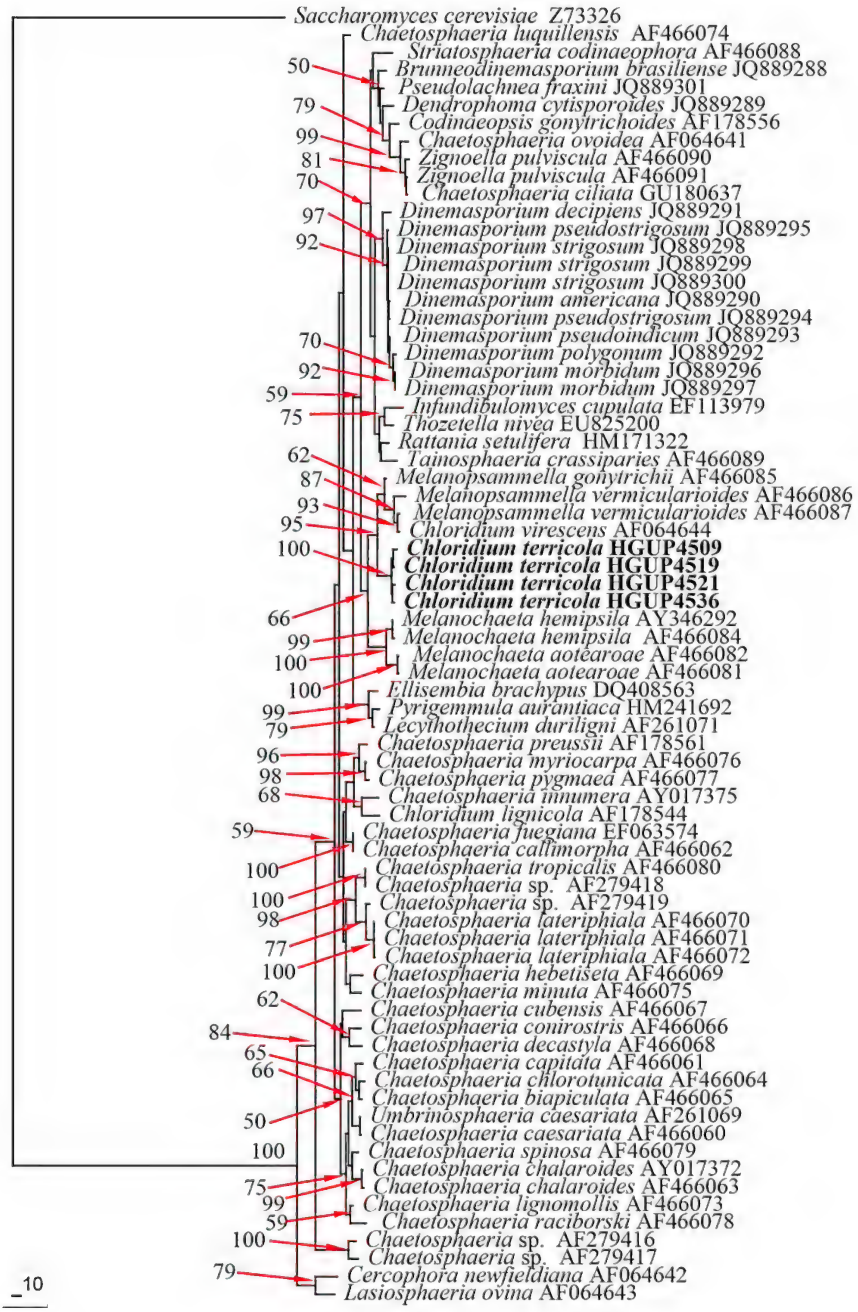


Fig. 2 (above). Topology showing the single most parsimonious tree, inferred from ITS sequence analysis. Bootstrap values less than 50% are not shown. The tree is rooted with *Cryptosporiopsis brunnea* (UAMH 10106). Type specimens are labeled with "T".

Fig. 3 (right). Topology showing the single most parsimonious tree, inferred from LSU sequence analysis. *Chloridium terricola* (in bold) clusters with *C. gonytrichii* (as *Melanopsammella*) and *C. viridescens* (shown also as *Melanopsammella vermicularioides*). Bootstrap values less than 50% are not shown. The tree is rooted with *Saccharomyces cerevisiae*.


Saccharomyces cerevisiae Z73326
Chaetosphaeria luquillensis AF466074
Striatosphaeria codinaeophora AF466088
Brunneodinemasporium brasiliense JQ889288
Pseudolachnea fraxini JQ889301
Dendrophoma cytisporoides JQ889289
Codinaeopsis gonytrichoides AF178556
Chaetosphaeria ovoidea AF064641
Zignoella pulviscula AF466090
Zignoella pulviscula AF466091
Chaetosphaeria ciliata GU180637
Dinemasporium decipiens JQ889291
Dinemasporium pseudostrigosum JQ889295
Dinemasporium strigosum JQ889298
Dinemasporium strigosum JQ889299
Dinemasporium strigosum JQ889300
Dinemasporium americana JQ889290
Dinemasporium pseudostrigosum JQ889294
Dinemasporium pseudoindicum JQ889293
Dinemasporium polygonum JQ889292
Dinemasporium morbidum JQ889296
Dinemasporium morbidum JQ889297
Infundibulomyces cupulata EF113979
Thozetella nivea EU825200
Rattania setulifera HM171322
Tainosphaeria crassiparies AF466089
Melanopsammella gonytrichii AF466085
Melanopsammella vermicularioides AF466086
Melanopsammella vermicularioides AF466087
Chloridium virescens AF064644
Chloridium terricola HGUP4509
Chloridium terricola HGUP4519
Chloridium terricola HGUP4521
Chloridium terricola HGUP4536
Melanochaeta hemipsila AY346292
Melanochaeta hemipsila AF466084
Melanochaeta aotearoae AF466082
Melanochaeta aotearoae AF466081
Ellisembia brachypus DQ408563
Pyrigemmula aurantiaca HM241692
Lecythothecium duriligni AF261071
Chaetosphaeria preussii AF178561
Chaetosphaeria myriocarpa AF466076
Chaetosphaeria pygmaea AF466077
Chaetosphaeria innumera AY017375
Chloridium lignicola AF178544
Chaetosphaeria fuegiana EF063574
Chaetosphaeria callimorpha AF466062
Chaetosphaeria tropicalis AF466080
Chaetosphaeria sp. AF279418
Chaetosphaeria sp. AF279419
Chaetosphaeria lateriphiala AF466070
Chaetosphaeria lateriphiala AF466071
Chaetosphaeria lateriphiala AF466072
Chaetosphaeria hebetiseta AF466069
Chaetosphaeria minuta AF466075
Chaetosphaeria cubensis AF466067
Chaetosphaeria conirostris AF466066
Chaetosphaeria decastyla AF466068
Chaetosphaeria capitata AF466061
Chaetosphaeria chlorotunicata AF466064
Chaetosphaeria biapiculata AF466065
Umbrinosphaeria caesariata AF261069
Chaetosphaeria caesariata AF466060
Chaetosphaeria spinosa AF466079
Chaetosphaeria chalaroides AY017372
Chaetosphaeria chalaroides AF466063
Chaetosphaeria lignomollis AF466073
Chaetosphaeria raciborski AF466078
Chaetosphaeria sp. AF279416
Chaetosphaeria sp. AF279417
Cercophora newfieldiana AF064642
Lasiosphaeria ovina AF064643
50
79
99
81
70
97
92
70
92
59
75
62
87
93
95
100
66
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10

Phylogenetic analysis

An NCBI-BLAST search of ITS sequences showed a 99% DNA identity between our isolates and *Phialocephala humicola* S.C. Jong & E.E. Davis and *P. xalapensis* Persiani & Maggi. We generated a phylogenetic tree of *Phialocephala* spp. based on ITS sequences (Fig. 2), from which we discovered that our taxon was close to, but not conspecific with, *P. humicola* and *P. xalapensis*.

To determine the taxonomic position of our isolates, we used a more inclusive analysis of the LSU region with *Saccharomyces cerevisiae* as outgroup (Crous et al. 2012) and ingroup sequences derived from Crous et al. (2012) and Fernández et al. (2006). Of the 1049 characters aligned, 297 are constant, 496 variable characters are parsimony-uninformative, and 256 are parsimony-informative. One of 32 most parsimonious trees with 1604 steps (CI = 0.610; HI = 0.390; RI = 0.723) derived from analysis of the LSU sequences is presented in Fig. 3. Our four *Chloridium terricola* sequences formed a branch with a 100% BS support (nearly without phylogenetic divergence). They were placed in *Chloridium* group with 100% BS, and showed a closer relationship with *Chloridium gonytrichii* (F.A. Fernández & Huhndorf) Réblová & Seifert and *C. virescens* (Pers.) W. Gams & Hol.-Jech. with high (95%) BS support.

Discussion

The International Code of Nomenclature for algae, fungi, and plants (McNeill et al. 2012) eliminated Art. 59, which in previous botanical codes permitted dual nomenclature for sexual and asexual stages in pleomorphic fungi. As noted above, Réblová et al. (2016) now accept *Melanopsammella* as a synonym of *Chloridium*. Fernández & Huhndorf (2005), who treated the genera before abandonment of Art. 59, noted that *Melanopsammella* species are easily distinguished by their small, one-septate ascospores that disarticulate into partial spores. Sexual stages are almost identical and separation of species is only possible by noting differences in the asexual stage. Because our isolates lacked a sexual stage, we base our morphological comparison on asexual characters.

The obvious morphological difference between *Chloridium terricola* and the asexual stage of *C. gonytrichii* (as *Melanopsammella* in Fernández & Huhndorf 2005) is the number of whorls of phialides: *C. terricola* has only one whorl of phialides (at the conidiophore apex), whereas *C. gonytrichii* has one apical whorl plus 5–8 additional whorls of phialides in the midsection of each conidiophore. Additionally, conidia are pale brown in *C. terricola* and light green conidia in *C.* (as *Melanopsammella*) *gonytrichii* (Fernández & Huhndorf 2005).

In comparison to *Chloridium terricola*, *C. virescens* (= *Melanopsammella vermicularioides*; see Fig. 3) conidia are hyaline, uni-/bi-guttulate and longer (3.2–4.0 × 2–2.5 µm; Gams & Holubová-Jechová 1976).

Phylogenetically, LSU sequence analysis clusters our taxon with (but taxonomically independent of) *C. gonytrichii* and *C. virescens*, supporting *Chloridium terricola* as a new taxon.

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Erysiphe russellii in Korea supported by new morphological and sequence analyses

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Abstract—Morphological and rDNA ITS sequence analyses were conducted on three strains obtained from leaf lesions on creeping wood sorrel (*Oxalis corniculata*) covered by a powdery mildew. No chasmothecia were observed, but the symptoms and traits of conidiophores and conidia agree well with those of *Erysiphe russellii* (previously recorded from Korea as *Microsphaera russellii*). Phylogenetic analysis shows that the three strains share 97.9-98.06 % identity with *Erysiphe pisi* (FJ378867) but form a separate *E. russellii* clade with a sequence of that species based on Japanese material. This supports the occurrence of *Erysiphe russellii* in Korea.

Key words—*Erysiphales,* pathogen, phylogeny, taxonomy

Introduction

Species in the genus *Erysiphe* (*Ascomycota*, *Erysiphales*, *Erysiphaceae*) are plant pathogens that cause powdery mildews (Takamatsu et al. 2015). The *Erysiphaceae* currently comprises about 873 species belonging to 16 genera (Braun & Cook 2012). It includes five tribes and two basal genera, which have been described by Mori et al. (2000) and Takamatsu et al. (2005). Creeping wood sorrel (*Oxalis corniculata, Oxalidaceae*) is a generally low-growing, spreading, perennial weedy or invasive plant with a yellow flower and rounded trifoliate clover-like leaves. It is widely distributed worldwide and occurs in diverse habitats, including arable fields and gardens or wastelands, but its origin is unknown. The leaves of the creeping wood sorrel are sometimes used as edible plant; it has a tangy taste due to a rich amount

Table 1. *Erysiphe* samples analyzed.

Species	Sample no.	Source (host)	Locality	GenBank ITS no.
E. diffusa	SMK15414	*Glycine soja*	South Korea	AB078812
	MUMH1162	*Glycine soja*	Japan	AB078813
	MUMH791	*Glycine max*	Japan	AB078800
	MUMH1463	*Glycine max*	USA	AB078810
	MUMH1451	*Glycine max*	Vietnam	AB078809
	MUMH56	*Amphicarpaea edgeworthii* var. *japonica*	NI	AB015934
E. glycines	MUMH52	*Desmodium oxyphyllum*	NI	AB015927
E. howeana	NI	*Oenothera biennis*	USA	AF011301
E. palczewskii	PMWI	*Caragana arborescens*	USA	GQ497277
	PMQB	*Caragana arborescens*	Canada	GQ497276
E. peruviana	DAG08-36	*Tecoma capensis*	USA	GU117107
	08-3618S	*Tecoma capensis*	USA	GU987124
E. pisi	FF 06	Pea	USA	FJ378867
	GH 07	Pea	USA	FJ378872
	GH 04	Pea	USA	FJ378870
	Lathyrus 07	Pea	USA	FJ378879
	GE 07	Pea	USA	FJ378869
	NI	*Lathyrus latifolius*	USA	AF011306
E. polygoni	bean 2A	*Phaseolus vulgaris*	Mexico	GU570595
	DNA14	*Rumex obtusifolius*	Japan	LC009892
	MUMH2431	*Polygonum* sp.	Argentina	LC010013
	UC1512295	*Polygonum arenastrum*	USA	AF011307
	UC1512308	*Rumex crispus*	USA	AF011308
E. sp. [1]	EB 2004	*Phaseolus vulgaris*	Brazil	AY739109
	DB 121211	*Oenothera biennis*	Italy	JQ288740
	3D	*Senna septentrionalis*	Mexico	JQ730709
***E. sp.* [2]**	**EML-COR2**	***Oxalis corniculata***	**Korea**	**KP941751**
	EML-COR3	***Oxalis corniculata***	**Korea**	**KP941752**
	MUMH0105	***Oxalis corniculata***	**Japan**	**LC009922**
E. trifoliorum	NI	*Trifolium repens*	China	JQ282732
	VPRI 22166	NI	NI	AF298542
	MUMH29s	*Trifolium vulgaris*	NI	AB015913

NI: Not identified.

Two unknown species are designated by [1] (still unknown) and [2] (supported as *E. russellii* after complete analysis).

of vitamin C and is traditionally used for drinks by infusing the leaves in hot water, sweetening, and then chilling.

Between July and October 2010 and in August 2012, symptoms of powdery mildew were observed on creeping wood sorrel plants in Suwon, Gwangju and Cheongyang in Korea. *Erysiphe russellii* (Clinton) U. Braun & S. Takam. has been reported from several species of *Oxalis* worldwide (Farr & Rossman 2015) and is the only powdery mildew that has been previously reported on creeping wood sorrel in Korea, based on morphology of the asexual morph. Therefore, the morphology of the new Korean powdery mildew collections on *Oxalis corniculata* has been re-examined, supplemented by a molecular phylogenetic analysis of rDNA ITS sequences, to confirm the identity of the causal agent of the powdery mildew as *E. russellii*. Molecular analyses of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) is a well-established method for fungal identifications at species level (White et al. 1990), which have been frequently applied to powdery mildews (Takamatsu et al. 1999, Cunnington et al. 2003).

Materials & methods

Sampling and morphological observations

Fresh samples infected by the powdery mildew pathogen were collected and examined by stereo and light microscopy. Voucher specimens were deposited in EML (Environmental Microbiology Laboratory) Fungarium (EMLF; Chonnam National University, Gwangju, Korea as EML-COR1 (obtained from a garden located in Suwon in July 2010), EML-COR2 (obtained from a pot for red pepper in Gwangju in August 2010), and EML-COR3 (obtained from a greenhouse located in Cheongyang in late October 2010).

DNA extraction, polymerase chain reaction amplification, and sequencing

Total genomic DNA was isolated from 50–100 mg of powdery mildew conidia and mycelia using the Accuprep® Genomic DNA Extraction Kit (Bioneer Corp., Daejeon, Korea). The rDNA ITS region was amplified using primers ITS1F and LR5F following Lee (2012a). The 20 µL PCR amplification mixture contained 2 µL DNA template, 1.5 µL per primer (5 pM/µL), 1 µL Accupower® PCR premix (containing Taq DNA polymerase, dNTPs, buffer, and tracking dye; Bioneer Corp.), and 14 µL of sterile water. Amplification parameters were an initial denaturation step at 95°C for 5 min, 30 cycles of denaturation at 94°C for 1 min + annealing at 54°C for 30 s + extension at 72°C for 1 min, and a final extension step at 72°C for 10 min. The PCR products were purified using the Accuprep® PCR Purification Kit (Bioneer Corp.). DNA sequencing was performed in an ABI 3700 Automated DNA sequencer (Applied Biosystems Inc.).

Phylogenetic analyses

After initial sequence alignment using CLUSTAL_X (Thompson et al. 1997), the alignment was edited manually. Sequence analyses were performed using MEGA 6 (Tamura et al. 2013) with the default settings. Phylogenetic trees were generated from the data using maximum-likelihood (ML), neighbor joining (NJ), and maximum parsimony (MP) methods. The Kimura 2-parameter model was selected as the best model to construct trees for NJ. Nearest-neighbor- interchange (NNI) was selected for the ML heuristic method, and the initial tree for ML was set automatically. The MP analysis was conducted with the heuristic search option using the tree-bisection-reconstruction (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting tree was tested with a bootstrap (BS) analysis using 1000 replications in the ML, NJ, and MP analyses. Tree scores, including tree length, consistency index (CI), and retention index (RI), were also calculated. *Erysiphe glycines* was used as outgroup. Sequences from this study and those from GenBank database are listed in Table 1.

Taxonomy

Erysiphe russellii (Clinton) U. Braun & S. Takam., Schlechtendalia 4: 13 (2000) Fig. 1

≡ *Microsphaera russellii* Clinton, in Peck, Rep. (Annual) New York Stat. Mus. 26: 80 (1874).

= *Oidium oxalidis* McAlpine, Proc. Roy. Soc. Victoria, N.S., 6: 219 (1894).

Mycelium on leaves, effuse, covering the entire leaf surface, white, thin, powdery. Hyphae somewhat straight to slightly wavy, septate, branched. Appressoria lobed and single or in opposite pairs. Conidiophores on top of hyphal mother cells, erect, 76.6–134 × 4.7–6.8 µm, with lecythiform to clavate apices above two or three cells. Conidia formed singly; primary conidia ellipsoidal to fusiform; secondary conidia, slender, cylindrical to oblong with slightly obtuse or truncate apices, 24–55 × 13.5–22 µm. Chasmothecia not observed.

Specimens examined—**KOREA**, Cheongyang, 36°27'45"N 126°51'34"E (EML-COR3), Gwangju, 35°10'0"N 126°55'0"E (EML-COR2), Suwon, 37°17'27"N 127°00'32"E (EML-COR1), on *Oxalis corniculata* L., October 2010, H.B. Lee. Voucher specimens (KOSPFG0000134896) were deposited in the herbarium of the National Institute of Biological Resources (NIBR), Incheon, Korea.

Discussion

In Korea, *Erysiphe russellii* was previously reported by Shin (2000, as *Microsphaera russellii* based on conidial morphology) as the causal pathogen on creeping wood sorrel. Previous studies (Takamatsu et al. 2003, 2015; Lee 2012a,b, 2013a,b, 2015; Lee et al. 2013) have successfully

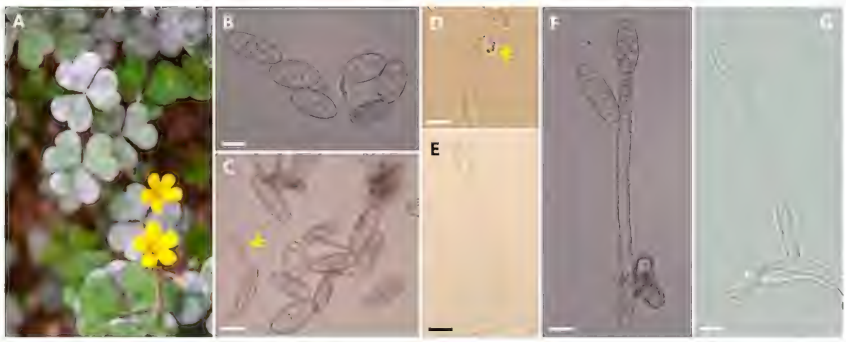

Fig. 1 Powdery mildew symptoms and the morphology of the causal pathogen, *Erysiphe* sp. EML-COR3 isolate. A. Powdery mildew symptoms on the leaves of creeping wood sorrel; B & C. Conidia and conidia with germ tube (yellow arrow); D. Appressoria on hyphae (yellow arrow). E-G. Conidiophores. Bars: B, D, F, G =20 μm; C, E=50 μm.

used rDNA ITS sequence data to identify *Erysiphe* species. Therefore, we conducted phylogenetic analysis based on three Korean strains and a single strain from Japan (LC009922) to confirm the identity of the Korean collections. ITS sequences from the three Korean strains were compared to those from related species retrieved from GenBank. Our sequence analysis (Fig. 2) clustered the sequences from strains EML-COR1, EML-COR2, and EML-COR3 together with a Japanese sequence in a separate clade in the phylogenetic tree assignable to *Erysiphe russellii*, supporting it as a distinct species. The morphology of the asexual morph most closely resembled that of *E. russellii* described by Braun (1987) and Shin (2000).

In the phylogenetic analyses, two small clades, highlighted by question marks emerged which could not be satisfactorily named (Fig. 2).

Various additional powdery mildew species have been reported on species of *Oxalis* worldwide, e.g. *Golovinomyces orontii* (Castagne) Heluta (= *Erysiphe polyphaga* Hammarl.), *Leveillula oxalidicola* T.Z. Liu & U. Braun, and a species of *Phyllactinia* (Braun & Cook 2012, Farr & Rossman 2015). This report is the first to verify the presence of *E. russellii* on creeping wood sorrel in Korea by DNA sequence comparisons. The pathogenicity of the biotrophic fungus was confirmed through inoculation experiments made by gently pressing infected leaves onto mature leaves of healthy creeping wood sorrel plants in a glasshouse. Seven days after inoculation, symptoms similar to those observed under natural infection were developed on the inoculated leaves of creeping wood sorrel plants (data not shown). More studies on

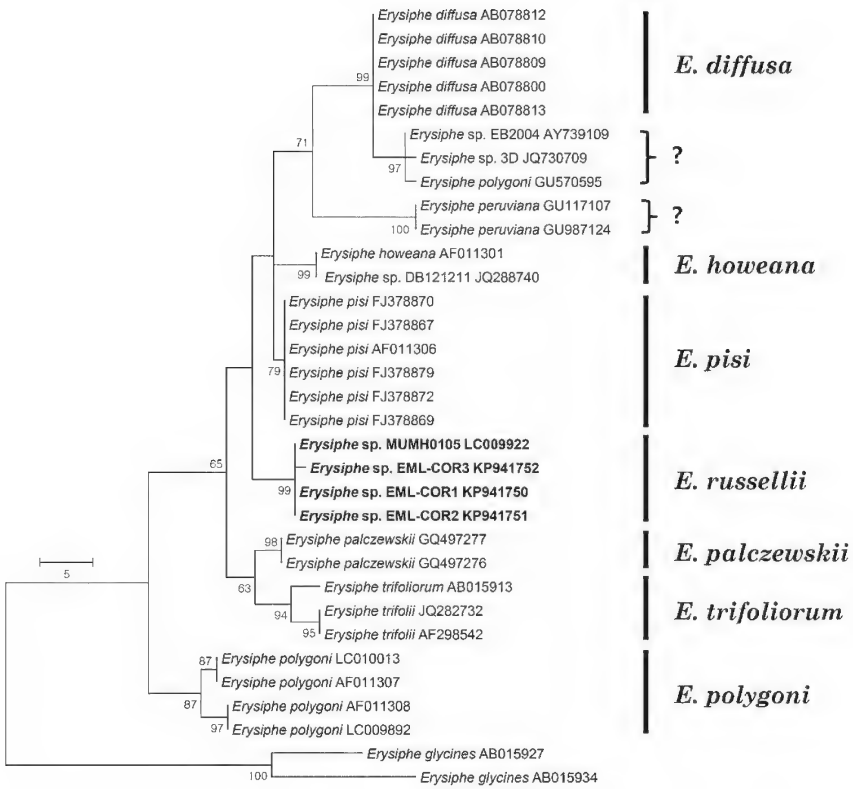

FIG. 2 Phylogenetic status of the EML-COR1, EML-COR2, and EML-COR3 and MUMH0105 isolates and related species based on maximum parsimony (MP) of the internal transcribed spacer (ITS) region sequences. Numbers at the nodes indicate the bootstrap values (>50 %) from 1000 replications. *Erysiphe glycines* was selected as outgroup. The thick line is based on a previous phylogenetic system constructed by Takamatsu et al. (2015). Two small clades, highlighted by question marks, emerged that could not be satisfactorily named. The scale bar indicates the number of nucleotide substitutions per site.

the distribution of powdery mildews on *Oxalis* in Korea, including a search for the sexual morph, are necessary. Moreover, sequence data based on North America samples (USA is the type locality of *E. russellii*) and material from Australia (type locality of *Oidium oxalidis*) are urgently required for comparison.

Acknowledgments

This work was supported by a grant from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE), Republic of Korea. Authors are grateful to Dr. Paul Kirk (Royal Botanic Garden at Kew, U.K.) and Dr. Jeffrey Stone (Oregon State University, Corvallis, USA) for kind review of the paper and to Prof. Dr Susumu Takamatsu (Mie University, Japan) for kind supply of sequence data of a Japanese strain.

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Phlebia brevibasidia sp. nov. from India

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Abstract—A new agaricomycetous species, *Phlebia brevibasidia*, is described from Punjab, India.

Key words—*Meruliaceae*, tropical, Anandpur Sahib, *Phlebia cremeoalutacea*, *Phlebia crassisubiculata*

During September 2015, Gurpreet and Avneet collected an unknown agaricomycetous fungus associated with an angiospermous log from Dabri in district Anandpur Sahib, Punjab, India. After comparison of macroscopic and microscopic characters (Rattan 1977; Eriksson et al. 1978, 1981; Bernicchia & Gorjón 2010; MycoBank 2016; Sharma 2012; Singh et al. 2010), it has been identified as a tropical species differentiated from temperate *Phlebia cremeoalutacea* and *P. crassisubiculata* by conspicuous morphological differences. A portion of the basidiocarp was sent to Dr. Nils Hallenberg (Denmark), who confirmed the findings. The holotype has been conserved in the Herbarium, Botany Department, Punjabi University, Patiala, India (PUN).

Phlebia brevibasidia G. Kaur, Avn.P. Singh & Dhingra, **sp. nov.** Plates 1, 2

MycoBank MB 816998

Differs from *Phlebia cremeoalutacea* by its bigger basidiospores, its stalked short basidia, its prominently projecting encrusted cystidia, and its compact pseudoparenchymatous context; and from *P. crassisubiculata* by its stalked short basidia and its thin subiculum.

Type: India, Punjab, Anandpur Sahib, Dabri, on angiospermous log, 30 July 2015, Gurpreet & Avneet 7932 (PUN, **holotype**).

Etymology: The epithet refers to short basidia

Basidiocarp resupinate, adnate, effused, ≤230 μm thick in section, hymenial surface smooth, orange white to pale orange to grayish orange when fresh, not changing much on drying; margins thinning, fibrillose, paler than the

Plate 1. *Phlebia brevibasidia* (holotype, PUN). 1. Basidiocarp showing hymenial surface.

color of the hymenial surface or indeterminate. Hyphal system monomitic. Generative hyphae clamped; subiculum ≤40 µm thick, of thick-walled, less branched, horizontal, ≤4 µm wide hyphae; context and subhymenium pseudoparenchymatous, ≤190 µm thick, of thin-walled, vertical, more branched, densely packed hyphae, ≤3.5 µm wide. Cystidia 58–101 × 12.8–17.5 µm, prominent, subfusiform, thick-walled, with basal clamp, encrusted with crystalline matter; projecting up to 50 µm out of hymenium. Basidia 15–18 × 5–7.3 µm, clavate, stalked, slightly thick-walled, 4-sterigmate, with basal clamp; sterigmata ≤3.8 µm long. Basidiospores 4.5–7.3 × 3.5–4.5 µm, ellipsoid, smooth, inamyloid, acyanophilous.

Remarks—*Phlebia cremeoalutacea* (Parmasto) K.H. Larss. & Hjortstam, a temperate species, differs in having subclavate–clavate basidia (>20 µm long), cystidia either enclosed and non-encrusted or projecting and encrusted, and smaller basidiospores (3–4.5 × 2–2.5 µm; Eriksson et al. 1981). Another temperate species, *P. crassisubiculata* Avn.P. Singh et al., differs in having longer, clavate basidia and a distinctly thicker subiculum (≤120 µm thick; Singh et al. 2010).

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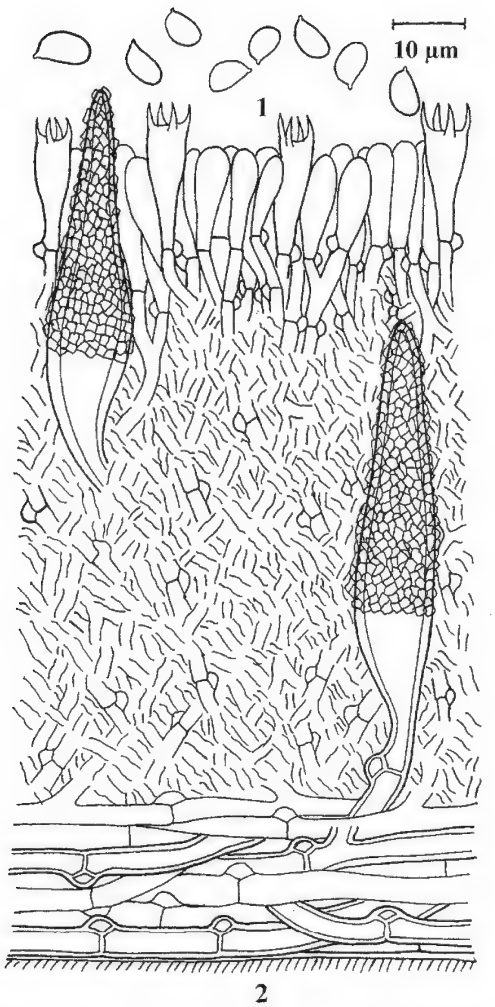

PLATE 2. *Phlebia brevibasidia* (holotype, PUN).
1. Basidiospores 2. Vertical section through basidiocarp.

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Two new records of puffballs in Thailand

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Abstract—*Calvatia holothurioides* and *Morganella puiggarii,* two puffballs in the *Agaricaceae,* are reported for the first time from Thailand. The specimens were identified based on morphological and ITS sequence analyses. Descriptions and illustrations are provided.

Key words—*Agaricales*, basidiomycete, phylogeny, taxonomy

Introduction

Puffballs in the genera *Bovista*, *Bovistella*, *Calvatia*, *Lycoperdon*, *Morganella*, *Tulostoma*, *Utraria*, and *Vascellum*, formerly classified as *Lycoperdaceae*, are now included in *Agaricaceae* (Kirk et al. 2008). Recent molecular phylogenetic studies have proposed a widening of *Lycoperdon* to incorporate traditional genera such as *Bovistella*, *Morganella*, *Utraria*, and *Vascellum* (Larsson & Jeppson 2008, Gube 2009). During a taxonomic survey of puffballs in northern Thailand, we collected specimens that corresponded to *Calvatia holothurioides* and *Morganella puiggarii,* species previously unknown in the country. Here we present the morphological characters and ITS sequence analyses of the Thai specimens.

Materials & methods

Morphology

Basidiomes were collected from Doi Suthep-Pui and Doi Inthanon National Parks, Chiang Mai Province, Thailand in 2013, and wrapped in aluminum foil or

placed in plastic specimen boxes for transport to the laboratory, where notes on macromorphological features and photographs were taken within 24 h. Color names and codes follow Kornerup & Wanscher (1978). The specimens were dried at 40–45 °C and deposited in the herbarium of the Research Laboratory for Excellence in Sustainable Development of Biological Resources, Faculty of Science, Chiang Mai University, Thailand (SDBR-CMU). Tissues from dried specimens were rehydrated in 95% ethanol followed by distilled water, 3% KOH, or Melzer's reagent for microscopical examination. Dimensions are based on at least 50 measurements of each anatomical structure. Scanning electron images of basidiospores were captured with a JEOL JSM-5910LV electron microscope.

DNA extraction & amplification

DNA was extracted from fresh specimens using a Favorgen® Genomic DNA Extraction Mini Kit. The internal transcribed spacer (ITS) region of ribosomal RNA gene was amplified by the polymerase chain reaction (PCR) with ITS4 and ITS5 primers under the following thermal conditions: 94 °C for 2 min; 35 cycles of 95 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min; and finally 72 °C for 10 min. PCR products were checked on 1% agarose gels stained with ethidium bromide under UV light and purified using NucleoSpin® Gel and PCR Clean-up Kit following the manufacturer's protocol. The purified PCR products were directly sequenced. Sanger sequencing was performed by 1ST Base Company (Kembangan, Malaysia) using the same ITS4 and ITS5 PCR primers. The new sequences were used to query GenBank database via BLAST (http://blast.ddbj.nig.ac.jp/top-e.html).

Phylogenetic analysis

A multiple alignment subroutine in Clustal X (Thompson et al. 1997) and phylogenetic tree was generated using the PAUP beta 10 vers. 4.0 (Swofford 2002). In maximum parsimony analysis, all characters were equally weighted and gaps were treated as missing data. Heuristic searches were performed with 100 random-addition sequence replicates and tree-bisection-reconnection branch swapping. Bootstrap analysis (Felsenstein 1985) was conducted with 1000 replicates using the same settings as above.

Taxonomy

Calvatia holothurioides Rebriev, Mikol. Fitopatol. 47: 21 (2013). Fig. 1

Basidiomes pyriform, turbinate to broadly excipuliform, 3–6 cm diam and 4–6 cm high. Exoperidium two-layered, brownish orange (6C6) to brown (7E4), slightly wrinkled, formed by hyaline, septate and branched hyphae, 4–7 µm wide, intermixed with hyaline, thin-walled vesicles 12–20 µm diam, with an underlying pseudoparenchymatous layer. Endoperidium light brown (7D6), formed by septate, thin-walled hyphae, 3–5 µm diam. Gleba cottony, yellowish white (4A2) when young, light brown (7D6) when mature. Subgleba light brown (7D6), composed of compacted cells. Paracapillitium

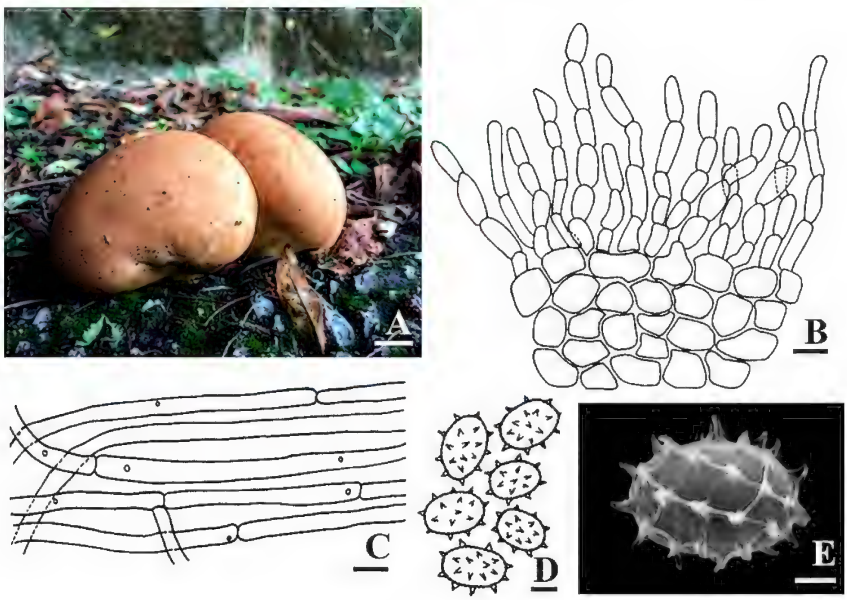

Fig. 1. *Calvatia holothurioides* (SDBR-CMU-NK0152). A: basidiomes; B: exoperidial layer; C: paracapillitial hyphae; D: basidiospores (LM); E: basidiospore (SEM). Scale bars: A = 10 mm, B = 10 μm, C = 5 μm, D = 2 μm, E = 1 μm.

thin-walled, septate, branched, hyaline, 3–5 μm diam. Pores roundish, ≤1.5 μm diam. Basidiospores ellipsoid to elongate, 3–5.5 × 2–3 μm (without ornamentation), Q = 1.5–1.8; hyaline in water and KOH, unchanging in Melzer's reagent; echinate, shortly pedicellate. Spines 0.2–0.5 μm long.

Specimen examined—**THAILAND, Chiang Mai Province, Muang District**, Doi Suthep-Pui National Park, 18°47′34″N 98°55′49″E, elevation 746 m, on soil in deciduous forest, 1 June 2013, Suwannarach N. & Kumla J. (SDBR-CMU-NK0152, GenBank KX064242).

Morganella puiggarii (Speg.) Kreisel & Dring, Feddes Repert 74: 116 (1967). Fig. 2

Basidiomes globose to subglobose, 0.6–2 cm diam. and 0.9–2.3 cm high, with whitish rhizomorphs attached at the base. Exoperidium white (3A1) to yellowish white (3A2) when fresh, dark brown (6F6) when dried, covered by minute conical tubercles, composed of chains of globose cells, 8–22 × 12–28 μm diam. Endoperidium soft, white (3A1). Gleba when young cottony and white (3A1), when mature powdery and dark brown (7F7). Pseudocolumella present. Subgleba orange-white (5A2), composed of compacted cells.

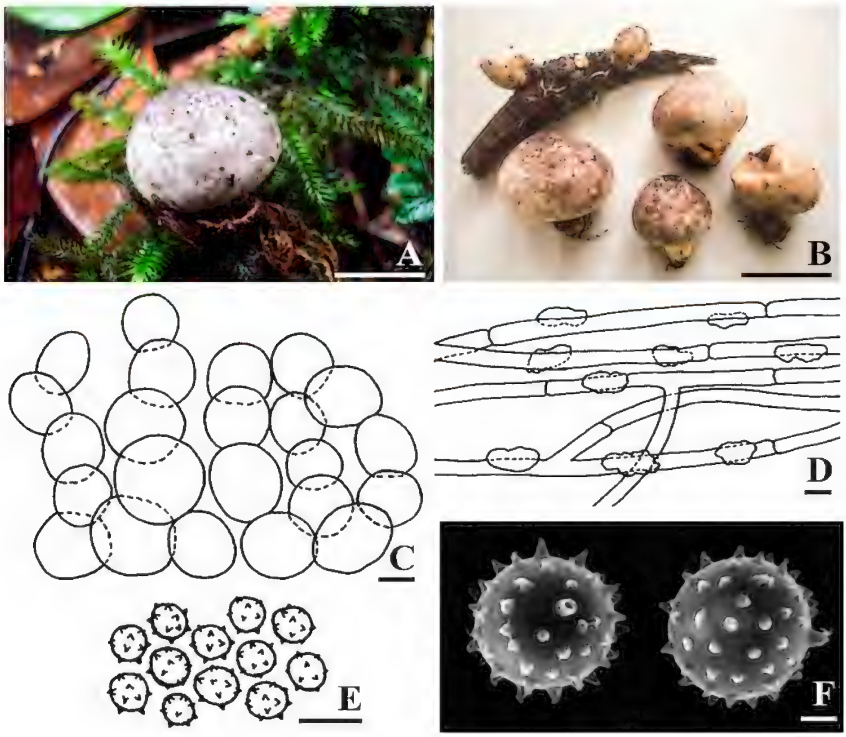

Fig. 2. *Morganella puiggarii* (SDBR-CMU-NK0141). A, B: basidiomes; C: exoperidial elements; D: paracapillitial hyphae; E: basidiospores (LM); F: basidiospores (SEM). Scale bars: A, B = 10 mm, C = 10 µm, D = 5 µm, E = 5 µm, F = 5 µm.

Paracapillitium thin-walled, 1–2.5 µm diam., septate, branched, hyaline, covered by an amorphous hyaline incrustation. Basidiospores globose, 3–4 µm diam. (without ornamentation), Q = 1–1.05; pale orange (5A3) in water and KOH, unchanging in Melzer's reagent; echinate, shortly pedicellate. Spines ≤0.5 µm long.

Specimen examined—**THAILAND, Chiang Mai Province, Chom Thong District**, Doi Inthanon National Park, 18°34′16″N 98°28′56″E, elevation 235 m, on rotting wood in mixed evergreen hill forest, 12 July 2013, Suwannarach N., Kumla J. & Lumyong S. (SDBR-CMU-NK0152, GenBank KX064241).

ITS sequence and phylogenetic analysis

The ITS sequences of *C. holothurioides* SDBR-CMU-NK0152 (659 bp) and *M. puiggarii* SDBR-CMU-NK0141 (699 bp) were deposited in GenBank. The

similarity search revealed a 98% similarity between SDBR-CMU-NK0152 and *C. holothurioides* JQ734547 and KJ909662 and a 96% similarity between SDBR-CMU-NK0141 and *M. purpurascens* (Berk. & M.A. Curtis) Kreisel & Dring KC414581.

In the phylogenetic analysis, the aligned dataset of 30 ITS sequences comprised 745 characters, of which 454 characters were constant, 76 variable characters were parsimony uninformative, and 215 characters were parsimony

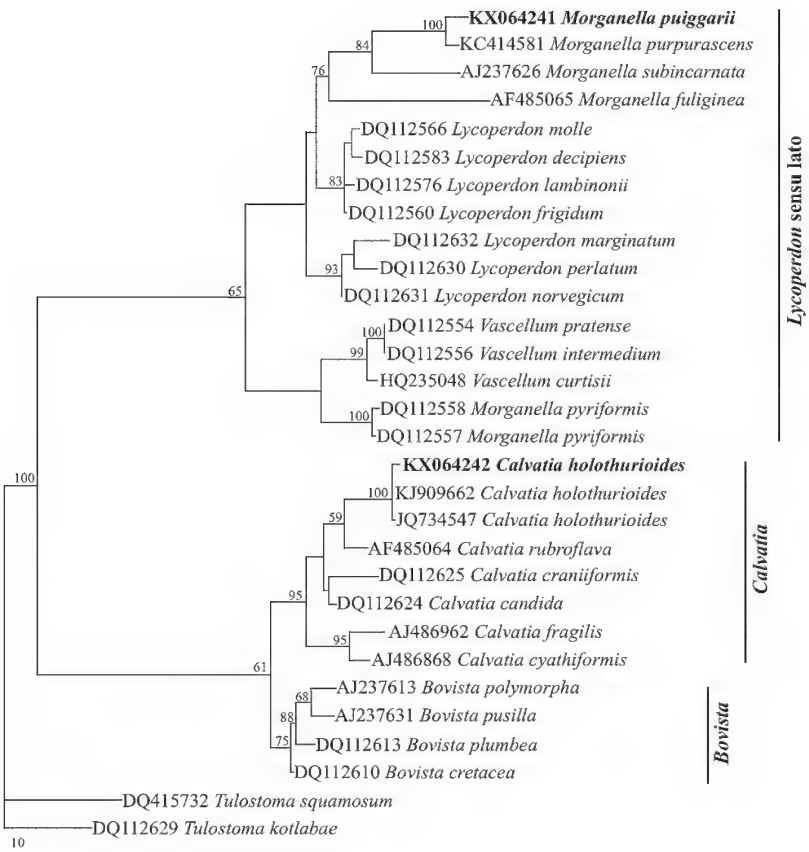

Fig. 3. One of 62 maximum parsimonious trees of 30 ITS sequences. *Tulostoma kotlabae* and *T. squamosum* were used as the outgroup. Numbers above branches show bootstrap supports ≥50%; scale bar represents ten substitutions per nucleotide position. The sequences obtained in this study are in bold.

informative. Heuristic searches produced a length of 667 steps, CI = 0.609, RI = 0.686, RC = 0.417 and HI = 0.391. One of 62 maximum parsimonious trees (Fig. 3) clustered our *C. holothurioides* ITS sequence with *C. holothurioides* sequences from GenBank within the *Calvatia* clade. Our sequence of *Morganella puiggarii* was assigned to the *Lycoperdon* sensu lato clade and formed a sister taxon to *M. purpurascens* with 100% bootstrap support.

Discussion

Calonge et al. (2003) assigned *C. holothurioides* to "*Calvatia* sect. *Sporocristata*" based on its echinulate and ellipsoidal spores. This invalid section contained only two species, *C. oblongispora* V.L. Suárez et al. and *C. sporocristata* Calonge (Calonge et al. 2003, Suárez et al. 2009). *Calvatia holothurioides* clearly differs from *C. oblongispora* and *C. sporocristata* by its pyriform to broadly excipuliform basidiomes and smaller spores (Calonge et al. 2003; Suárez et al. 2009; Rebriev 2013). *Calvatia holothurioides* has been reported previously from Vietnam (Rebriev 2013).

Morganella puiggarii differs from *M. arenicola* Alfredo & Baseia, *M. albostipitata* Baseia & Alfredo, *M. benjaminii* (Rick) Cortez et al., *M. compacta* (G. Cunn.) Kreisel & Dring, *M. fuliginea* (Berk. & M.A. Curtis) Kreisel & Dring, *M. nuda* Alfredo & Baseia, *M. stercoraria* P. Ponce de León, *M. rimosa* Baseia & Alfredo, *M. sulcatostoma* C.R. Alves & Cortez, and *M. velutina* (Berk. & M.A. Curtis) Kreisel & Dring by its tuberculate exoperidium (Ponce de León 1971; Cortez et al. 2007; Alfredo et al. 2012, 2014; Alves & Cortez 2013, 2014). The presence of an eucapillitium in *M. pyriformis* (Schaeff.) Kreisel & D. Krüger clearly separates this species from *M. puiggarii*. The paracapillitium of *M. samoensis* (Bres. & Pat) P. Ponce de León and *M. subincarnata* (Peck) Kreisel & Dring is wider than of *M. puiggarii* (Ponce de León 1971). *Morganella puiggarii* forms a sister taxon to *M. purpurascens*, the latter having a pitted endoperidium (Ponce de León 1971). Moreover, *M. puiggarii* was previously known from Brazil and Paraguay (Ponce de León 1971).

The combination of morphological and molecular features supports *C. holothurioides* and *M. puiggarii* as new records for Thailand. Prior to this study, there have been no reports of *Calvatia* "sect. *Sporocristata*" and only four *Morganella* species (*M. compacta*, *M. fuliginea*, *M. purpurascens* and *M. pyriformis*) recorded from the country (Phanichapol 1968, Ellingsen 1982, Kasuya et al. 2006, Chandrasrikul et al. 2011, Kumla et al. 2014).

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Cyanodermella asteris sp. nov. (*Ostropales*) from the inflorescence axis of *Aster tataricus*

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Abstract—An endophytic fungus isolated from the inflorescence axis of *Aster tataricus* is proposed as a new species. Phylogenetic analyses based on sequences from the ribosomal DNA cluster (the ITS1+5.8S+ITS2, 18S, and 28S regions) and the RPB2 gene revealed a relationship between the unknown fungus and the *Stictidaceae* lineage of the *Ostropales.* The new species, *Cyanodermella asteris,* grows in standard fungal growth media as a fluffy, pink filamentous fungus. Asexual and sexual sporulation has not yet been observed on media or in the plant.

Key words—*Ascomycota, Asteraceae, Lecanoromycetes, Pezizomycotina*

Introduction

Aster tataricus (*Asteraceae*) is native to northern Asia in Siberia, Mongolia, Japan, Korea, and China. Its roots are well known in traditional Chinese and Japanese medicine due to their diuretic, antibacterial, antiviral, and anti-ulcer activities (Shao et al. 1997a, b; Shirota et al. 1997). Diverse secondary metabolites have been identified in *A. tataricus* roots: shiononе-type triterpenes [e.g., astataricusones and astataricusol (Zhou et al. 2013); astershiononеs (Zhou et al. 2014)], cyclopeptides[e.g., astins (Morita et al. 1996, Xu et al. 2013)], and flavonoids [e.g., quercetin and kaempferol (Wang et al. 2003)].

With over 2700 species in eleven families, the *Ostropales* (Hibbett et al. 2007) represent a large part of the *Lecanoromycetes* (*Ascomycota*, *Pezizomycotina*). Although most ostropalean fungi are lichenized, parasites and saprotrophs are also known (Grube & Hawksworth 2007; Lutzoni et al. 2004; Sherwood 1977a, 1977b; Wedin et al. 2004, 2006). Baloch et al. (2010), who outline the current taxonomy of the *Ostropales*, note that most *Stictidaceae* are saprotrophs and form very small fruiting bodies.

Here we describe our isolation of an endophytic fungus from the inflorescence of *Aster tataricus* and demonstrate its placement in the *Ostropales*. DNA sequences from the newly discovered fungus matched no previously sequenced species, although it did show phylogenetic affinities with *Cyanodermella*. For that reason, we propose this endophyte as a new species, named here *Cyanodermella asteris*.

Materials & methods

Chemicals were purchased from Duchefa (Haarlem, The Netherlands) and Roth (Karlsruhe, Germany). Plant hormones were obtained from Duchefa and Sigma-Aldrich (Hamburg, Germany) and PCR reagents from Thermo Fisher Scientific (Waltham, USA).

Collecting & field sites

The *Aster tataricus* host plant was obtained from Sarastro Stauden (Ort im Innkreis, Austria) and cultivated in the Plant Physiology laboratory greenhouse, Technische Universität Dresden, Germany. The cultivar is henceforth referred to as *A. tataricus* cv. Austria.

Isolation

The fungus was isolated from surface-sterilized inflorescence axes of *Aster tataricus* cv. Austria. After the axes were treated first for 30 s with 70% ethanol + 0.1% Triton X-100, then for 5–7 min with 4.2% sodium hypochlorite + 0.1% Triton X-100, and washed 3 times with autoclaved distilled water, samples were cultivated

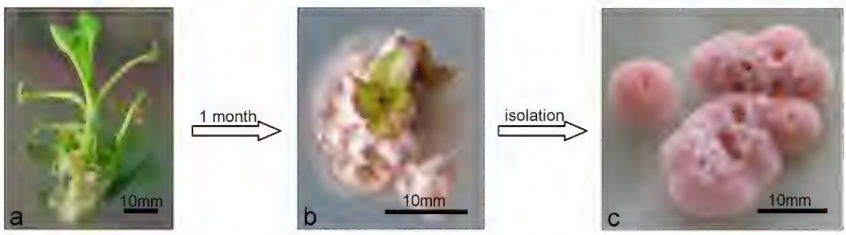


Fig. 1. Isolation of the new endophyte *Cyanodermella asteris*. a. During the first few weeks, the new endophyte *C. asteris* was isolated from sterile plantlets growing on hormone containing MS medium. b. Antimycotic treatment did not reduce the fungal outgrowth, and the fungus still appeared on the plant surface and grew on the MS medium. c. Isolation and cultivation on potato dextrose agar produced a fluffy pink filamentous fungus.

on Murashige-Skoog (MS) medium (Murashige-Skoog 1962; 4.4 g/l MS medium including vitamins, 3% sucrose, 1% phyto agar, pH 5.8) including auxins and/or cytokinins (i.e., naphtylacetic acid, indole-3-butyric acid, 6-benzylaminopurine) usually used to stimulate sterile plants in vitro. The MS plates were incubated in long day cycles (16 h light, 23°C; 8 h dark, 18°C). After several transfers of sterile plantlets to fresh MS plates, we found a fungus growing from the small plants into the MS medium. Initially considered a contaminant, the fungus was treated with antimycotics (50 µg/ml Nystatin® for 4 months and penicillin/ streptomycin/ amphotericin B solution for 1 month). However, the treatment did not eliminate the fungus from the plant culture. In order to identify the fungus, we isolated hyphae from the plantlets for cultivation on potato dextrose (26.5 g/l potato dextrose bouillon, 1% phyto agar, pH 5.8) and malt extract agar (3% malt extract, 1% phyto agar, pH 5.8) in an incubator in the dark at 28°C (Fig.1).

DNA extraction, PCR amplification, and sequencing

DNA was extracted from mycelium grown in potato dextrose broth following a modified protocol by Möller et al (1992) and precipitated with isopropanol. The mixture was left overnight at –20°C, spun down, and the DNA pellet washed three times with 70% ethanol to ensure salt-free DNA. The DNA was amplified using Phusion® polymerase according to the LifeTechnologies instruction manual. The primers (0.5 µM) used for both PCR and sequencing were ITS1F & ITS4 and NS3 & NS8 (White et al. 1990), LROR & LR7 (Vilgalys & Hester 1990), and fRPB2-5F, -7cR, -7cF, & -11aR (Liu et al. 1999). PCR conditions were initial denaturation at 98°C for 30 s, 35 cycles of 98°C for 10 s, appropriate annealing temperature for 30 s, and 72°C for 1 min followed by a final elongation at 72°C for 5 min. Amplification was carried out in an Eppendorf Mastercycler ep gradient S. PCR products were separated on a 1xTAE gel, cut out, extracted using a GE Healthcare Gel Extraction Kit, and sequenced at Eurofins MWG (Ebersberg, Germany).

Total DNA was isolated from plants as described above and checked with the appropriate fungal primers under the same PCR conditions except for an annealing temperature of 68°C. The PCR fragments were also sequenced and compared with the known rDNA sequence of the endophytic fungus to confirm the presence of the fungus in the plants.

Phylogenetic analysis

Sequences of the nuclear internal transcribed spacer region (ITS, including ITS1+5.8S rDNA+ITS2), the large (28S) and small (18S) nuclear rDNA subunits, and the second largest RNA polymerase II (RPB2) subunit from *Cyanodermella asteris* were run in a BLASTn search (Altschul et al. 1990). The different DNA regions were also used to build a phylogeny including closely related fungi. For this, the ITS, 18S, 28S and RPB2 sequences from *C. asteris* and other fungi representing the *Stictidaceae* (Table 1) were aligned using the Guidance2 Server (Landan & Graur 2008, Penn et al. 2010, Sela et al. 2015) with the default settings (including MAFFT algorithm) and *Phlyctidaceae* and *Coenogoniaceae* species as out-group. After sequence alignment, phylogenetic trees were generated from single region and combined sequences using MEGA6.0 (Tamura et al. 2013). The phylogenetic trees were inferred using the Maximum Likelihood method based on the General Time Reversible (Nei & Kumar 2000) or the Kimura-2-parameter model (Kimura 1980). Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The bootstrap method was used with 1000 replicates (Felsenstein 1985).

The combined alignment and phylogenetic tree do not include all species, because rDNA cluster sequences were incomplete for some (Table 1).

Microscopy

The fungus was examined under an AP-8 binocular microscope (Thalheim Spezial Optik, Pulsnitz, Germany) and tissue samples were inspected with transmitted light with an Axiolab microscope (Zeiss, Jena, Germany). Additionally, fungal hyphae from a shake culture were stained with 1 g Congo Red in 100 ml 5% SDS to visualize fungal cell walls and septa (Clémençon 2009) under the microscope.

Growth tests

Malt extract broth (50 ml) was inoculated with 0.5 ml of homogenized hyphae of *Cyanodermella asteris* and incubated at 180 rpm. The medium was buffered in the pH test with 50 mM phosphate or 50 mM citrate buffer and cultures were incubated at 22°C for 10 d. The cultures of the temperature test were incubated in unbuffered medium (pH 5.3) for 17 d. Growth rates (mm/day) were recorded from 42 d old cultures on potato dextrose and malt extract agar plates kept at 23°C in the dark. Colors were coded according to Munsell (1905).

TABLE 1. DNA sequences from *Stictidaceae* used for phylogenetic trees

SPECIES	* = ISOLATE ^ = VOUCHER	18S	ITS	28S	RPB2
Absconditella lignicola	*EB211	—	FJ904669.1	FJ904669.1	—
	Svensson & Baloch SW187 (S)	—	—	—	HM244776.1
A. rubra	^van den Boom 52517	KT454800.1	KT454800.1	—	—
A. sp.	*Spribille 39168	KR017250.1	KR017125.1	KR017188.1	KR017501.1
	^GS5_2_17	—	KF128882.1	—	—
	^Palice 3820	—	—	AY300825.1	—
A. sphagnorum	*M24	EU940022.1	JX298897.1	EU940095.1	EU940311.1
Acarosporina microspora	*AFTOL 78	AY584667.1	DQ782834.1	AY584643.1	AY584682.1
Bryodiscus arctoalpinus	^Baloch SW057	—	—	HM244760.1	HM244781.1
Carestiella socia	*GG2410	—	AY661687.1	AY661687.1	—
	^Wedin 7194 (UPS)	—	—	—	HM244782.1
Coenogonium leprieurii	^Kauff pa04021998-522	AF465457.1	—	AF465442.1	AY641032.1
C. luteum	^Ryan 31430 (ASU)	AF279386.1	—	AF279387.1	AY641038.1
	*AFTOL 352	—	HQ650710.1	—	—
C. pineti	^HB Palice	—	—	AY300834.1	—
	^Thor 19164 (UPS)	—	—	—	HM244786.1
Conotrema populorum	^UME41471	U86582.1	—	—	—
	*GG2610a	—	AY527327.1	—	—
	^Gilenstam 2353 (UPS)	—	—	AY300833.1	—
C. sp.	*MW7200		AY527313.1	AY527313.1	—
	*AN3222	—	AY527336.1	AY527336.1	—
Cryptodiscus foveolaris	*EB155	—	FJ904673.1	—	—
	*GG2603a	—	—	AY661683.1	—
	^Baloch & Arup SW166 (S)	—	—	—	HM244787.1
C. gloeocapsa	*EB93	FJ904696.1	FJ904674.1	—	—
	^Tibell 23543 (UPS)	—	—	—	HM244788.1
C. incolor	*S:F116574	—	NR_121357	—	—
C. pallidus	*EB152	—	FJ904679.1	FJ904679.1	—
	^Baloch & Wedin SW174 (S)	—	—	—	HM244789.1
C. pini	^Baloch & Arup SW175 (S)	—	HM244762.1	HM244762.1	HM244790.1
C. rhopaloides	*EB100	—	FJ904685.1	—	HM244791.1
*C. sp.*uncultured	clone F9	—	KP323396.1	—	—
Cyanodermella asteris	**strain 03HOR06-2-4**	**KT758843**	**KT758843**	**KT758843**	**KU934214**

Species	* = isolate ^ = voucher	18S	ITS	28S	RPB2
C. oleoligni	strain DTO 301-G1	KX999145.1	KX950434.1	KX950461.1	KX999147.1
C. viridula	^E. & C. Baloch SW129 (S)	—	—	HM244763.1	HM244792.1
	^UME29146	U86583.1	—	—	—
Glomerobolus gelineus	*AFTOL 1349	—	DQ247782.1	—	DQ247793.1
	strain JK 5548K	DQ247811.1	—	—	—
	strain JK 5584C	—	—	DQ247798.1	—
Odontotrema phacidiellum	^Gilenstam 2625 (UPS)	—	—	HM244769.1	HM244802.1
O. phacidioides	^Palice 11440 (S)	—	—	HM244770.1	HM244803.1
Ostropa barbara	*AFTOL 77	AY584666.1	—	AY584642.1	—
	^Wedin & Baloch SW071 (S)	—	HM244773.1	—	HM244806.1
Petractis luetkemuelleri	^Nimis & Tretiach 2000 (TSB 31659)	AF465461.1	—	AF465454.1	—
	*AFTOL 381	—	HQ650714.1	—	—
	^Geletti & Tretiach 1995 (TBS)	—	—	—	AY641061.1
P. nodispora	^A.Orange 17559 (NMW [C.2007.001.282])	FJ588712.1	—	FJ588713.1	—
	*NMW C.2007.001.284	—	NR_120312.1	—	—
Phlyctis agelaea		—	—	HQ659177.1	—
	^PHLAGE08257	—	—	—	KC020280.1
P. argena	*AFTOL 1375	DQ986725.1	—	DQ986771.1	DQ992458.1
	*BP8	—	KJ409433.1	—	—
Schizoxylon albescens	^ Gilenstam 2696a (UPS)	DQ401142.1	NR_121319.1	DQ401144.1	—
	^Wedin 7919 (UPS)	—	—	—	HM244813.1
S. sp.	*GG2365	—	AY661689.1	AY661689.1	—
Stictis brunnescens	*GG2359	—	AY661688.1	AY661688.1	—
	^Wedin 7651 (UPS)	—	—	—	HM244814.1
S. confusa	^Wedin 7070 (UPS)	—	DQ401143.1	DQ401143.1	HM244815.1
S. populorum	^UME41471	U86582.1	—	—	—
	*MW7301	—	AY527334.1	—	—
	^Gilenstam 2353 (UPS)	—	—	AY300833.1	—
	^Wedin 7626 (UPS)	—	—	—	HM244817.1
S. radiata	^Palice (ESS 21520)	AY300864.1	—	—	—
	*AFTOL 398	—	DQ782846.1	—	—
	^Jamie Platt JP222	—	—	AF356663.1	AY641079.1
S. sp.	*GG2445a	—	AY527318.1	AY527318.1	—
	*GG2440b	—	AY527321.1	—	—
	*GG2620b	—	AY527332.1	AY527332.1	—
S. urceolatum	*AFTOL 96	DQ983488.1	HQ650601.1	—	DQ992478.1

Taxonomy

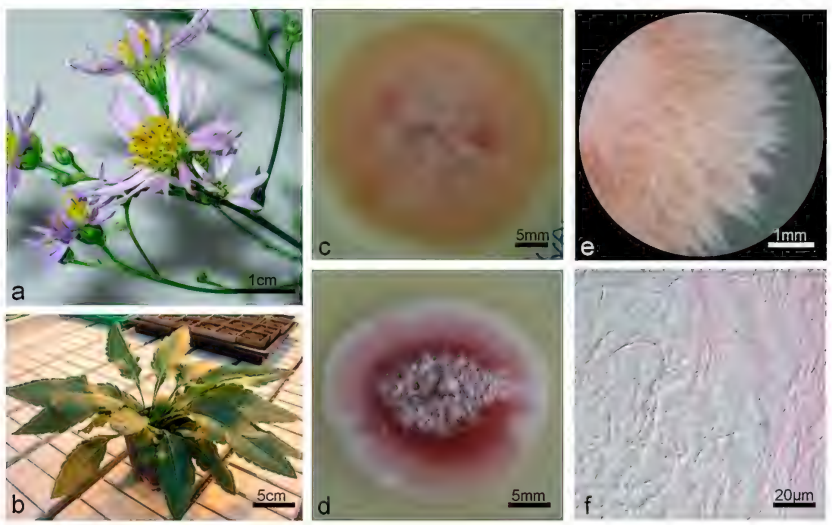


Fig. 2. The endophytic fungus *Cyanodermella asteris*. a, b. Habitus of the host *Aster tataricus* showing inflorescences and ground rosette; c. 55 d-old growth on malt extract agar; d. 55 d-old growth on potato dextrose agar; e. filamentous structure of the colony (36 d old); f. hyphae stained with Congo Red.

Cyanodermella asteris L. Jahn & Ludw.-Müll. **sp. nov.** Fig. 2

MycoBank MB 814158

Differs from *C. viridula* by its endophytic habit and its rose-pink hyphae in culture.

Type—Germany, Saxony, Dresden, isolated as endophyte of *Aster tataricus* cv. Austria, 21 Mar 2013, L. Jahn, **holotype** Herb. DR 043292 (metabolically inactive), living culture at DSMZ under DSM 100826.

Etymology—The epithet 'asteris' is derived from the host plant, *Aster tataricus* cv. Austria.

Sexual stage unknown. Colonies (14 d old) on potato dextrose agar white (N 9) to pale pink (2.5R 9/2); in age fading from pale pink to light pink (2.5R 8/6); after 30 days, becoming white towards the margin and turning light and deep pink (2.5R 6/10) to vivid red (5.0R 4/14) towards the center. Colors less intense on malt extract agar: 7–14 d old colonies usually colorless (N 9) and hardly distinguishable from the medium, >50 d old colonies colorless at the margin and light pink at the center.

Growth rate 0.7 ± 0.3 mm/day on potato dextrose agar and 0.9 ± 0.1mm/day on malt extract agar at an optimum temperature of 22–24°C and a pH of 5.8–6.

Host & distribution—The distribution of the endophytic fungus *C. asteris* may be linked to the distribution of its host, *A. tataricus,* which is native to northern Asia. *Cyanodermella asteris* has been isolated and detected by PCR in several plants of *A. tataricus* cv. Austria and also detected by PCR in *A. tataricus* plants obtained from the Botanical Garden Dresden, Germany, but which originated in Siberia. Isolation of *C. asteris* from these plants is currently in progress.

Phylogenetic results

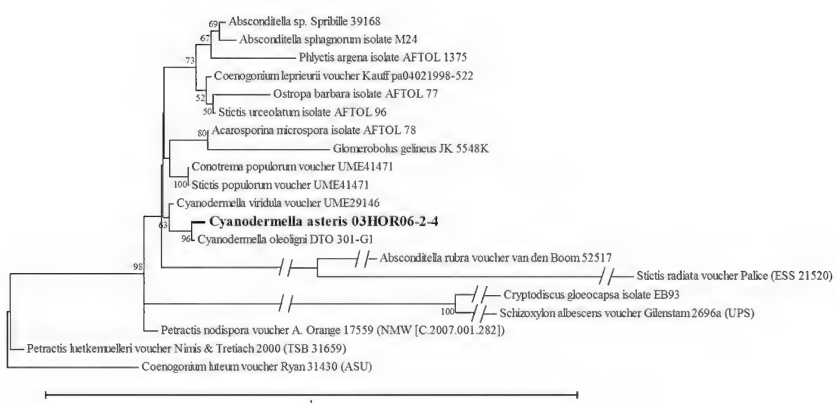
The nuclear rDNA sequences of *Cyanodermella asteris* used for identification (as the "rDNA cluster") comprised the 18S (1539 bp), ITS (ca. 535 bp) and 28S (1359 bp) (GenBank KT758843). The first BLASTn search showed a distant relationship to the *Stictidaceae* lineage of the ostropalean fungi (Table 2). This led to a second BLASTn search against the *Stictidaceae* (Table 3). The 18S *C. asteris* sequence showed as nearest neighbor to *C. oleoligni* (98% identity) and *C. viridula* (95% identity), *Acarosporina microspora* (94% identity), and less closely related *Stictis* species. Similarly, the 28S *C. asteris* sequence revealed a relationship with *C. oleoligni* (94% identity) and *C. viridula* (95% identity). The ITS region showed a closest match to *C. oleoligni* (91% identity), *Stictis* sp. (96% identity), and *Carestiella socia* (96% identity); no ITS sequences were available for *C. viridula.*

Table 2. BLASTn results of the *C. asteris* rDNA cluster region

	C. asteris			BLASTn hits			
Loci	# BPs	GC %	Species	accession #	query cover	ident	E value
18S	1521	48.0	*Cyanodermella oleoligni*	KX999145.1	74%	98%	0.0
			Trapelia involuta	AF119499.2	78%	91%	0.0
			Trapelia placodioides	AF119500.2	78%	95%	0.0
			Acarospora smaragdula	AY552543.1	78%	91%	0.0
			Cyanodermella viridula	U86583.1	78%	91%	0.0
ITS	899	53.9	*Pleopsidium chlorophanum*	DQ525472.1	47%	84%	$4*10^{-97}$
			Acarospora insignis	LN890273.1	39%	87%	$6*10^{-95}$
			Acarospora smaragdula	EU870652.1	38%	86%	$4*10^{-97}$
28S	1329	49.4	*Cyanodermella oleoligni*	KX950461.1	89%	94%	0.0
			Umbilicaria crustulosa	HM161593.1	100%	90%	0.0
			Umbilicaria haplocarpa	HM161534.1	99%	90%	0.0

TABLE 3. BLASTn results of the *C. asteris* rDNA cluster region against *Stictidaceae*

LOCI	SPECIES	BLASTN HITS			
		ACCESSION #	QUERY COVER	IDENT	E VALUE
18S	*Cyanodermella oleoligni*	KX999145.1	74%	98%	0.0
	Cyanodermella viridula	U86583.1	77%	95%	0.0
	Acarosporina microspora	AY584667.1	76%	94%	0.0
	Stictis urceolatum	DQ983488.1	76%	94%	0.0
ITS	*Cyanodermella oleoligni*	KX950434.1	32%	91%	$2*10^{-95}$
	Stictis radiata	AY527308.1	30%	88%	$3*10^{-88}$
	Stictis brunnescens	AY661688.1	20%	96%	$3*10^{-84}$
	Cryptodiscus pini	FJ904682.1	28%	88%	$4*10^{-82}$
	Carestiella socia	AY661682.1	20%	96%	$4*10^{-82}$
28S	*Cyanodermella oleoligni*	KX950461.1	89%	94%	0.0
	Xyloschistes platytropa	KJ766680.1	97%	88%	0.0
	Stictis radiata	AY340575.1	77%	90%	0.0
	Acarosporina microspore	AY584643.1	83%	90%	0.0
	Cyanodermella viridula	HM244763.1	41%	95%	0.0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Absconditella sphagnorum isolate M24
Phlyctis argena isolate AFTOL 1375
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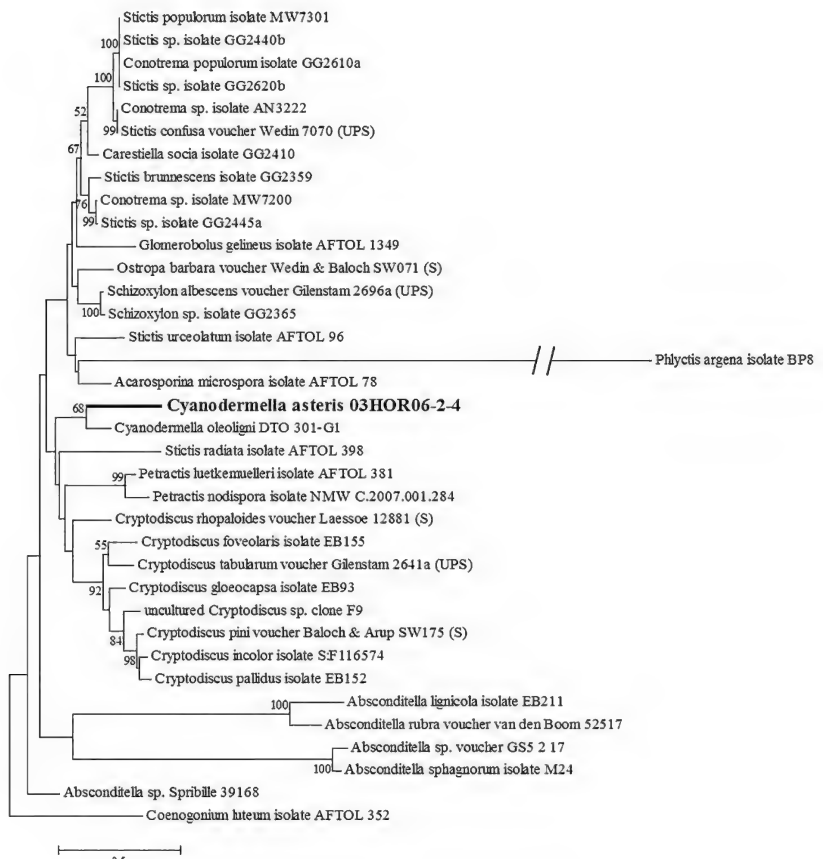
Ostropa barbara isolate AFTOL 77
Stictis urceolatum isolate AFTOL 96
Acarosporina microspora isolate AFTOL 78
Glomerobolus gelineus JK 5548K
Conotrema populorum voucher UME41471
Stictis populorum voucher UME41471
Cyanodermella viridula voucher UME29146
Cyanodermella asteris 03HOR06-2-4
Cyanodermella oleoligni DTO 301-G1
Absconditella rubra voucher van den Boom 52517
Stictis radiata voucher Palice (ESS 21520)
Cryptodiscus gloeocapsa isolate EB93
Schizoxylon albescens voucher Gilenstam 2696a (UPS)
Petractis nodispora voucher A. Orange 17559 (NMW [C.2007.001.282])
Petractis luetkemuelleri voucher Nimis & Tretiach 2000 (TSB 31659)
Coenogonium luteum voucher Ryan 31430 (ASU)
1

FIG. 3. 18S sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the Kimura-2-parameter model (Kimura 1980). The tree with the highest log likelihood (–15822.6420) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.6859)]. The percentage of trees clustering the associated taxa is shown next to the branches. *Coenogoniaceae* and *Phlyctidaceae* were used as out-group.

The RPB2 sequence (2059 bp, GenBank KU934214) was used to refine phylogenetic relationships within the *Stictidaceae*. Phylogenetic analyses based on the individual 18S, ITS, 28S, and RPB2 sequences of *Cyanodermella asteris* with other *Stictidaceae* strongly support a sister-relationship among *C. asteris, C. viridula,* and *C. oleoligni* (Figs 3–6). The combined ML tree also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Stictis sp. isolate GG2440b
Conotrema populorum isolate GG2610a
Stictis sp. isolate GG2620b
Conotrema sp. isolate AN3222
Stictis confusa voucher Wedin 7070 (UPS)
Carestiella socia isolate GG2410
Stictis brunnescens isolate GG2359
Conotrema sp. isolate MW7200
Stictis sp. isolate GG2445a
Glomerobolus gelineus isolate AFTOL 1349
Ostropa barbara voucher Wedin & Baloch SW071 (S)
Schizoxylon albescens voucher Gilenstam 2696a (UPS)
Schizoxylon sp. isolate GG2365
Stictis urceolatum isolate AFTOL 96
Phlyctis argena isolate BP8
Acarosporina microspora isolate AFTOL 78
Cyanodermella asteris 03HOR06-2-4
Cyanodermella oleoligni DTO 301-G1
Stictis radiata isolate AFTOL 398
Petractis luetkemuelleri isolate AFTOL 381
Petractis nodispora isolate NMW C.2007.001.284
Cryptodiscus rhopaloides voucher Laessoe 12881 (S)
Cryptodiscus foveolaris isolate EB155
Cryptodiscus tabularum voucher Gilenstam 2641a (UPS)
Cryptodiscus gloeocapsa isolate EB93
uncultured Cryptodiscus sp. clone F9
Cryptodiscus pini voucher Baloch & Arup SW175 (S)
Cryptodiscus incolor isolate S:F116574
Cryptodiscus pallidus isolate EB152
Absconditella lignicola isolate EB211
Absconditella rubra voucher van den Boom 52517
Absconditella sp. voucher GS5 2 17
Absconditella sphagnorum isolate M24
Absconditella sp. Spribille 39168
Coenogonium luteum isolate AFTOL 352
0.5

Fig. 4. ITS sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–11371.6910) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 1.4100)]. The percentage of trees clustering the associated taxa is shown next to the branches. *Coenogoniaceae* were used as out-group.

clusters *C. asteris* with the other two *Cyanodermella* species, but the combined phylogenetic tree is not as well supported as the others due to missing sequences (Fig. 7; Treebase TB2:S18310).

Discussion

Sequence analyses of the nuclear rDNA cluster from *Cyanodermella asteris* implied a close relationship with the *Stictidaceae* lineage of the *Ostropales* (*Lecanoromycetes*, *Pezizomycotina*, *Ascomycota*). The *Stictidaceae* are either saprotrophic or lichenized, and no stictidaceous species have been described as plant endophytes. However, Schulz & Boyle (2005) noted that

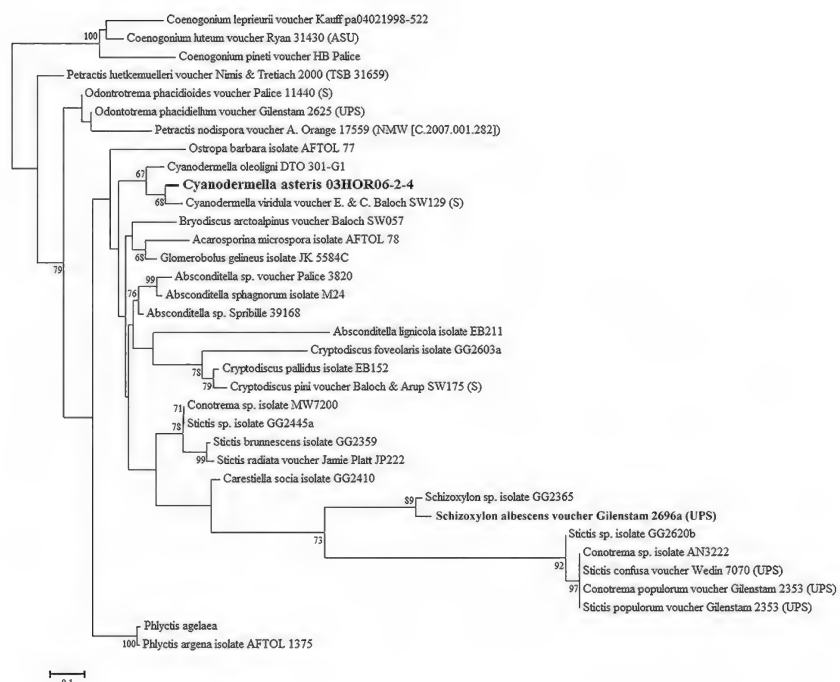


Fig. 5. 28S sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–15372.4003) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.6449)]. The percentage of trees clustering the associated taxa is shown next to the branches. *Coenogoniaceae* and *Phlyctidaceae* were used as out-group.

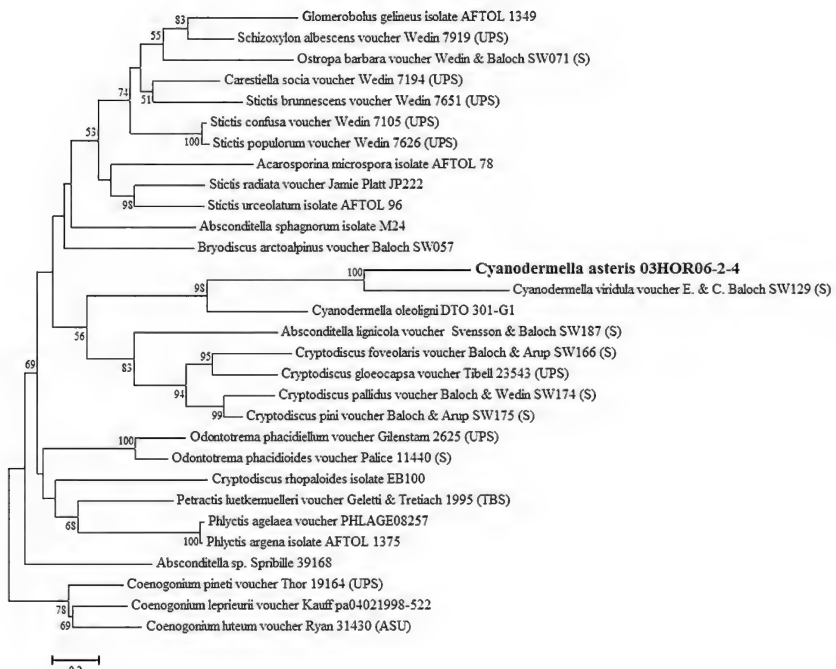
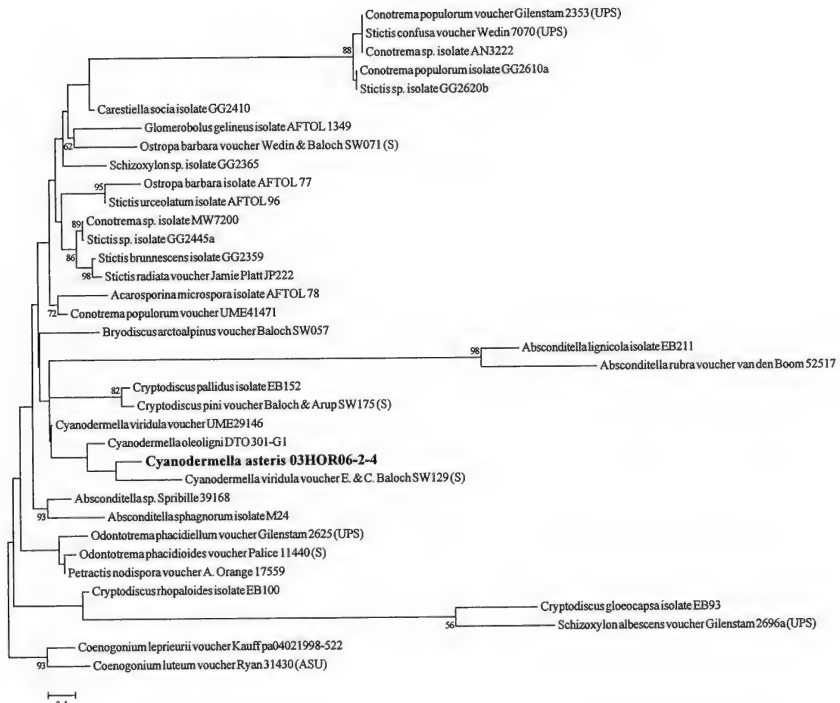

Glomerobolus gelineus isolate AFTOL 1349
Schizoxylon albescens voucher Wedin 7919 (UPS)
Ostropa barbara voucher Wedin & Baloch SW071 (S)
Carestiella socia voucher Wedin 7194 (UPS)
Stictis brunnescens voucher Wedin 7651 (UPS)
Stictis confusa voucher Wedin 7105 (UPS)
Stictis populorum voucher Wedin 7626 (UPS)
Acarosporina microspora isolate AFTOL 78
Stictis radiata voucher Jamie Platt JP222
Stictis urceolatum isolate AFTOL 96
Absconditella sphagnorum isolate M24
Bryodiscus arctoalpinus voucher Baloch SW057
Cyanodermella asteris 03HOR06-2-4
Cyanodermella viridula voucher E. & C. Baloch SW129 (S)
Cyanodermella oleoligni DTO 301-G1
Absconditella lignicola voucher Svensson & Baloch SW187 (S)
Cryptodiscus foveolaris voucher Baloch & Arup SW166 (S)
Cryptodiscus gloeocapsa voucher Tibell 23543 (UPS)
Cryptodiscus pallidus voucher Baloch & Wedin SW174 (S)
Cryptodiscus pini voucher Baloch & Arup SW175 (S)
Odontotrema phacidiellum voucher Gilenstam 2625 (UPS)
Odontotrema phacidioides voucher Palice 11440 (S)
Cryptodiscus rhopaloides isolate EB100
Petractis luetkemuelleri voucher Geletti & Tretiach 1995 (TBS)
Phlyctis agelaea voucher PHLAGE08257
Phlyctis argena isolate AFTOL 1375
Absconditella sp. Spribille 39168
Coenogonium pineti voucher Thor 19164 (UPS)
Coenogonium leprieurii voucher Kauff pa04021998-522
Coenogonium luteum voucher Ryan 31430 (ASU)
0.2

Fig. 6. RPB2 sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–29390.8544) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5486)]. The percentage of trees clustering the associated taxa together is shown next to the branches. *Coenogoniaceae* and *Phlyctidaceae* were used as outgroup.

many endophytes are facultative in that they adopt a saprotrophic strategy as soon as their host plant dies. Although *C. asteris* did not visibly develop any fruit bodies or other structures on dying and dead plant material of *Aster tataricus,* a saprotrophic habit cannot be excluded.

The species within the *Stictidaceae* live in different ecological niches over the whole world (see Sherwood 1977a, b, Eriksson 1967, 1981, Wedin et al. 2006, Czarnota, Kukwa 2008, Baloch et al. 2009; Fig. 8). Nonetheless, no *Stictidaceae* have been recorded from Siberia or Mongolia. Our species is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Conotrema populorum isolate GG2610a
Stictis sp. isolate GG2620b
Carestiella socia isolate GG2410
Glomerobolus gelineus isolate AFTOL 1349
Ostropa barbara voucher Wedin & Baloch SW071 (S)
Schizoxylon sp. isolate GG2365
Ostropa barbara isolate AFTOL 77
Stictis urceolatum isolate AFTOL 96
Conotrema sp. isolate MW7200
Stictis sp. isolate GG2445a
Stictis brunnescens isolate GG2359
Stictis radiata voucher Jamie Platt JP222
Acarosporina microspora isolate AFTOL 78
Conotrema populorum voucher UME41471
Bryodiscus arctoalpinus voucher Baloch SW057
Absconditella lignicola isolate EB211
Absconditella rubra voucher van den Boom 52517
Cryptodiscus pallidus isolate EB152
Cryptodiscus pini voucher Baloch & Arup SW175 (S)
Cyanodermella viridula voucher UME29146
Cyanodermella oleoligni DTO 301-G1
Cyanodermella asteris 03HOR06-2-4
Cyanodermella viridula voucher E. & C. Baloch SW129 (S)
Absconditella sp. Spribille 39168
Absconditella sphagnorum isolate M24
Odontotrema phacidiellum voucher Gilenstam 2625 (UPS)
Odontotrema phacidioides voucher Palice 11440 (S)
Petractis nodispora voucher A. Orange 17559
Cryptodiscus rhopaloides isolate EB100
Cryptodiscus gloeocapsa isolate EB93
Schizoxylon albescens voucher Gilenstam 2696a (UPS)
Coenogonium leprieurii voucher Kauff pa04021998-522
Coenogonium luteum voucher Ryan 31430 (ASU)
0.1

FIG. 7. Combined rDNA cluster + RPB2 sequence-based phylogeny of *Cyanodermella asteris* and closely related taxa in the *Stictidaceae* using Maximum Composite Likelihood based on the General Time Reversible model (Nei & Kumar 2000). The tree with the highest log likelihood (–50824.7554) is shown. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.6150)]. The percentage of trees clustering the associated taxa together is shown next to the branches. *Coenogoniaceae* were used as out-group.

both the first to inhabit a plant with origins in northern Asia and the first isolated endophyte representing the *Stictidaceae*.

It should be noted that within the *Ostropales,* the *Stictidaceae* are poorly sampled with relatively few sequences available to generate a phylogeny. The nearest neighbors of *C. asteris* were identified as *C. viridula* (Berk. & M.A. Curtis) O.E. Erikss. and *C. oleoligni* van Nieuwenhuijzen & Samson. The only sequences available for *C. viridula*—the 18S and 28S—show a 94% identity match with those from *C. asteris*. The more diverse ITS region—usually used

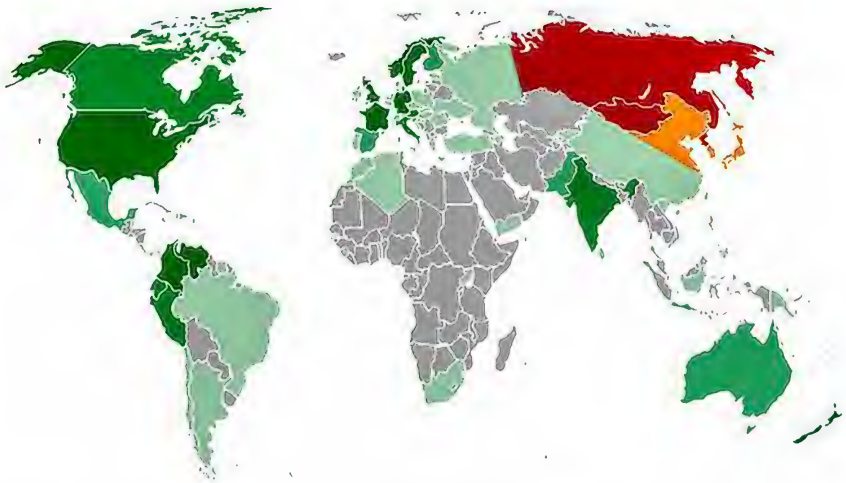

Fig. 8. Worldwide distribution of the *Stictidaceae* and *Aster tataricus* (Sherwood 1977a, b, Eriksson 1967, 1981, Wedin et al. 2006, Czarnota, Kukwa 2008, Baloch et al. 2009, Flann 2009). *Aster tataricus* is native to northern Asia including Siberia, Mongolia, Japan, Korea, and northern China, from which only a few *Stictidaceae* are described. Legend: Areas with >15 (●), 10–14 (●), 5–9 (●), or 1–4 (●) different species of *Stictidaceae;* native distribution of *Aster tataricus* (●); overlapping areas (●) and (●) are coded (●).

to identify and classify unknown fungal species (Schoch et al. 2012)—is not yet published from *C. viridula*. *Cyanodermella oleoligni* shows slightly higher similarities with *C. asteris* than *C. viridula*.

Cyanodermella is characterized by its perithecoid apothecia (Höhnel 1919; Eriksson 1967, 1981), which are very small and closed (Baloch et al. 2010). Only three species are described so far: *C. oleoligni, C. viridula*, and *C. candida* (Setch.) O.E. Erikss.

Cyanodermella viridula is known from old leaves of *Leymus arenarius* on beaches found in Sweden and western Russia near St. Petersburg as well as on twigs in North America, while *C. candida* is known only on ferns in Mexico (Eriksson 1967, 1981). *Cyanodermella oleoligni* (van Nieuwenhuijzen et al. 2016), which was isolated from oil-treated timber of *Pinus sylvestris,* grows slowly in grey to green colonies on fungal media. With the sexual stage of *C. asteris* not yet known despite an intensive search for ascomata on living and dead plant material, we expect its classification to be refined as more sequences from the *Stictidaceae* become available.

The role of *C. asteris* in or for the host plant is not yet clear. Because no endophyte-free cultivars of *A. tataricus* are available to us, we are unable to determine whether the fungus aids the plant's growth in the environment. Schulz & Boyle (2005) and Rodriguez et al. (2009) report that many endophytic fungi do not promote growth even when the plant is under stress. We speculate that if host plant serves as a habitat to protect *C. asteris* from the environment, the need for the fungus to adjust to the host plant might explain the initially slow growth (later somewhat accelerated; data not shown) of *C. asteris* in culture media. Future research will enable us to determine which metabolic and other features are characteristic for *C. asteris.*

Acknowledgments

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Eight *Caloplaca* species newly recorded from Bolivia, including *C. crocina* comb. nov.

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Abstract—Eight species of *Caloplaca* s. lat. are reported as new to Bolivia: *Caloplaca baueri*, *C. cinnabarina*, *C. crocina*, *C. darbishirei*, *C. ochraceofulva*, *C squamosa*, *C. subsoluta*, and *C. texana*. *Caloplaca texana* is also reported as new for Peru and the Southern Hemisphere. The new combination *Caloplaca crocina* is proposed and compared with other *Caloplaca* spp. producing hourglass-shaped ascospores. Taxonomic notes and data on the distribution and habitat preferences are presented for all treated species.

Key words—lichenized fungi, *Ascomycota*, *Lecanoromycetes*, *Teloschistaceae*, tropical dry forest

Introduction

Classification of the family *Teloschistaceae* has been the subject of intensive research over the last few years (e.g. Gaya et al. 2012, Arup et al. 2013, Kondratyuk et al. 2013). In the modern classification proposed by Arup et al. (2013), 39 genera in *Teloschistaceae*, with more than 1000 species estimated. The former *Caloplaca*, the most species-rich genus within *Teloschistaceae,* has been split into many genera based on phylogenetic DNA analyses. Research on *Teloschistaceae* systematics is still in progress and authors themselves (Arup et al. 2013, Kondratyuk et al. 2013) agree that the taxonomy is still not settled. As South American *Caloplaca* species are poorly known, especially at the molecular level, we decided not to apply the new systematics in the current paper and temporarily retain the species in *Caloplaca* s. lat. (see also note in Materials & methods).

Teloschistaceae are represented by five genera in Bolivia: *Caloplaca*, *Josefpoeltia*, *Teloschistes*, *Xanthomendoza*, and *Xanthoria* (Feuerer 2015). Only six *Caloplaca* taxa have been reported from the country: *C. brebissonii* (Fée) J. Sant. ex Hafellner & Poelt [≡ *Lecanora brebissonii*], *C. cerina* var. *chloroleuca* (Sm.) Th. Fr., *C. cirrochroa* (Ach.) Th. Fr. [≡ *Placodium cirrochroum*], *C. crocea* (Kremp.) Hafellner & Poelt subsp. *crocea* [also as *Callopisma xanthaspis*], *C. holocarpa* (Ach.) A.E. Wade s. lat. [also as *Lecanora pyracea*], and *C. quadrilocularis* (Nyl.) Zahlbr. [≡ *Lecidea quadrilocularis*] (Nylander 1859, 1861; Malme 1926; Hafellner & Poelt 1979; Feuerer et al. 1998; Rodriguez Flakus et al. 2014). There are a few South American countries in which the knowledge of *Caloplaca* is relatively comprehensive, e.g., Argentina (57 species), Brazil (52), Chile (45), and Uruguay (30) (Feuerer 2015). Based on checklists by Feuerer (2015), there are c. 140 *Caloplaca* species in all of South America.

Caloplaca s. lat. is fairly well understood in Europe, Asia, North America, Australasia, and both polar regions, while it remains poorly understood in Africa and South America. However, data on South American *Caloplaca* are included in several taxonomic studies (e.g., Zahlbruckner 1917, 1924; Malme 1926, Magnusson 1950; Hafellner & Poelt 1979;, Kärnefelt 1990; Wetmore & Kärnefelt 1998, 1999; Kärnefelt et al. 2002; Scutari et al. 2002; Rosato & Arup 2010; Lumbsch et al. 2011; Søchting & Sancho 2012; Vargas Castillo & Beck 2012; Kondratyuk et al. 2014; Søchting et al. 2014), regional floristic surveys (e.g. Nylander 1859, 1861; Zahlbruckner 1933; Dodge 1936; Follmann 1967; Osorio 1983; Feuerer et al. 1998; Aptroot 2015), and checklists (e.g. Calvelo & Liberatore 2002). The work by Malme (1926) deserves special attention, since it includes descriptions of 34 South American species and a key for their identification.

This study is part of a project concerning the taxonomic evaluation of the genus *Caloplaca* s. lat. in Bolivia. Our main purpose is to determine taxonomic diversity and phylogenetic relationships of recognized species. We also intend to analyze the distribution and habitat preferences of individual species.

This paper covers eight species of *Teloschistaceae* newly recorded for Bolivia: *Caloplaca baueri*, *C. cinnabarina*, *C. crocina*, *C. darbishirei*, *C. ochraceofulva*, *C. squamosa*, *C. subsoluta*, and *C. texana*. *Caloplaca texana* is also reported as new to Peru and the Southern Hemisphere. *Caloplaca crocina* is compared with eight *Caloplaca* species producing ascospores with thickened spore walls: *C. calcitrapa*, *C. cupulata*, *C. dichroa*, *C. haematommona*, *C. lobulascens*, *C. pollinii*, *C. rheinigera*, and *C. yuennana*. Taxonomic notes and data on the distribution and habitat preferences for all eight species are presented.

Materials & methods

Collections—The authors examined their own collections and herbarium specimens from B, KRAM, LD, MEL, S. Both authors collected specimens in the years 2004–15 throughout Bolivia (depts.: Beni, Chuquisaca, Cochabamba, La Paz, Potosi, Santa Cruz, Tarija) in the following vegetation zones (Ibisch & Mérida 2004): high mountain vegetation, Tucumano-Boliviano montane forest, semi-desert Inter-Andean Valleys, Chaqueño and Chiquitano dry forests, savanna Moxos, and anthropogenic woodlands. Flakus also collected some material from Colca Valley, Peru. The voucher specimens are available at KRAM and/or LPB.

Morphological examination—The materials were studied using standard microscopic techniques. Macro-morphological characters were measured from dry material. Hand-cut sections and squash preparations mounted in water were examined and measured microscopically, and the granulation of anatomical structures was observed in polarized light. Solubility of granules/crystals and color reactions were determined using 25% KOH (K) and 65% nitric acid (N). Hydrochloric acid (HCl) was used to test the presence of calcium carbonate ($CaC0_3$) in the substrate.

Nomenclature—In the most recent classification of *Teloschistaceae*, Arup et al. (2013) and Kondratyuk et al. (2013) transferred five species treated here to new genera: *Caloplaca darbishirei* to *Austroplaca* Søchting et al.; *C. squamosa* and *C. subsoluta* to *Squamulea* Arup et al.; *C. texana* to *Wetmoreana* Arup et al.; and *C. cinnabarina* to *Brownliella* S.Y. Kondr. The other three featured species and some other species discussed here do not have modern recombinations.

New combination

Caloplaca crocina (Kremp.) K. Wilk & R. Vargas, **comb. nov.** Plate 1

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≡ *Lecidea crocina* Kremp., Flora 61: 519. 1878.
Type: Argentina, 1873–1874, Lorentz & Hieronymus (M!, **holotype**).

≡ *Blastenia crocina* (Kremp.) Müll. Arg., Rev. Mycol. (Toulouse) 10: 68. 1888.

≡ *Lecanora crocina* (Kremp.) Stizenb., Ber. Thätigk. St. Gallischen Naturwiss. Ges. 1893–94: 235. 1895.

≡ *Callopisma crocinum* (Kremp.) Malme, Ark. Bot. 20A(9): 19. 1926.

≡ *Placodium crocinum* (Kremp.) Räsänen, Borbásia 1: 129. 1939.

"*Caloplaca crocina*" Marcelli (as "*crocinum*"), nom. inval.

Thallus not or sometimes slightly parasitized, thin to endophloeic, or thick, 60–150 µm thick; crustose, continuous to rimose, forming irregular patches, creamy, sometimes with greenish tinge, epruinose, smooth to slightly rough (when parasitized); vegetative propagules absent; prothallus usually present, conspicuous, black. Cortex poorly developed, amorphous, hyaline, anthraquinones absent, K–, N–; algal layer discontinuous, algae concentrated

in groups. Apothecia always present, abundant, scattered or crowded in small groups of 2–3, round, angular or flexuous when compressed, adnate, zeorine to almost biatorine, 0.1–1.0(–1.4) mm wide; disc first concave, then ±plane, rarely slightly convex in old apothecia, orange to brownish orange or olive; proper margin swollen and prominent in young apothecia, then thinning and only slightly prominent, yellow orange, often contrasting against the disc, epruinose; thalline margin conspicuous or inconspicuous to almost absent, in young apothecia hidden at base of apothecia or invisible, in mature and old apothecia generally better developed, although often remaining ±hidden at base of apothecia, fragmented (as tiger stripes). Parathecium thick, ≤200 µm, prosoplectenchymatous and composed of irregularly orientated hyphae (chondroid hyphae), anthraquinones present; amphithecium usually strongly reduced, algae scarce and in distinct groups (only occasionally abundant), cortex absent or amorphous, anthraquinones absent; epihymenium yellow red or yellow brown, granular (anthraquinones), K+ purple; hymenium (65–)75–100(–120) µm thick; hypothecium yellowish or pale brownish, K–, oil droplets present or absent, prosoplectenchymatous, stipe present. Paraphyses simple or forked, 1.5–2.0 µm broad with upper cell not or slightly wider, 2–3 µm, oil droplets absent. Asci 8-spored. Ascospores hyaline, hourglass-shaped, 15–24 × 7–10(–13) µm, septum (3–)4–5(–6) µm, apical spore walls 1.5–3.0 µm.

Pycnidia not observed.

Specimens examined: ***Caloplaca crocina***—**BOLIVIA. Dept. Beni, Prov. Ballivian**: near Reyes village, Moxos savanna, 14°18′10″S 67°18′49″W, alt. 192 m, on tree bark, 29 Nov. 2004, Wilk 2507 (KRAM), Flakus 4380 (KRAM, LPB), and 5 Dec 2004, Wilk 2320 (KRAM, LPB); Reyes village, 14°17′54″S 67°20′05″W, alt. 189 m, on wooden fence, good insolation, 29 Nov. 2004, Wilk 2332 (KRAM). **Prov. Cercado**, Trinidad, Missones Guarayos, alt. 250 m, Sept. 1926, E. Werdermann 2490 (S, B). **Dept. La Paz, Prov. Franz Tamayo**, Parque National y Área Natural de Manejo Integrado Madidi, Mojos, along the path to Virgen del Rosario, 14°33′22″S 68°52′27″W, alt. 1635 m, submontane anthropogenic woodlands, sunny and dry place, 31 Oct. 2007, Wilk 9356, 9380 (LPB), 9367, 9370, 9478 (KRAM). **Dept. Santa Cruz, Prov. Cordillera**, Parque Nacional y Área Natural de Manejo Integrado Kaa-Iya del Gran Chaco: gasoducto Bolivia-Brasil, between Tucavaca colony and National Park border, 18°31′40″S 60°41′26″W, alt. 314 m, Chiquitano forest, 4 Dec. 2010, Flakus 19218 (KRAM, LPB); gasoducto Bolivia-Brasil KP 164, 60 km of Tucavaca colony, 18°27′29″S 61°23′01″W, alt. 292 m, Chaqueño forest, 3 Dec. 2010, Flakus 18960 (LPB), 18961 (KRAM); Charrata village near campamento de guardaparques, 18°28′05″S 62°05′43″W, alt. 307 m, Chaqueño forest, 2 Dec. 2010, Flakus 20144 (KRAM, LPB). **Prov. Chiquitos**, near Santa Cruz de la Vieja, mirador a San José de Chiquitos village, 17°52′21″S 60°45′41″W, alt. 501 m, Cerrado de la Chiquitanía (wooded savannas), 5 Dec. 2010, Flakus 19280 (LPB). **Dept. Tarija, Prov. Aniceto Arce**, Serranía de Propiedad Arnold, 22°13′19″S 64°33′41″W, alt. 1309 m, Tucumano-Boliviano montane forest, 24 Dec. 2010, Flakus 18866/1 (LPB). **Dept. Chuquisaca, Prov. Luis Calvo**, Parque Nacional y Área Natural de Manejo Integrado Serranía del

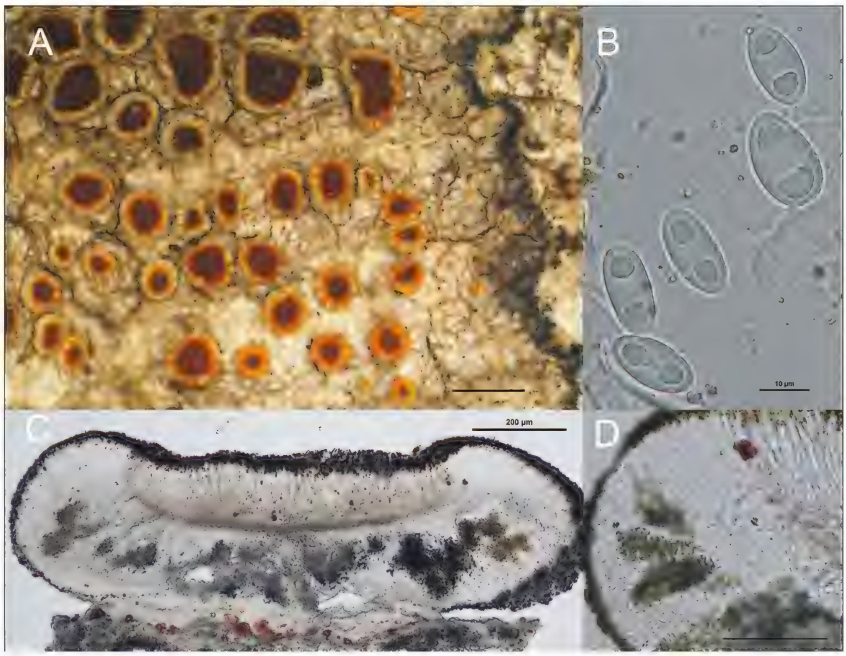

Plate 1. *Caloplaca crocina*: A—habit (Wilk 2507, KRAM); scale bar= 1 mm. B—ascospores (Wilk 9380, LPB); scale bar= 10 µm. C—vertical section of apothecium (Wilk 9478, KRAM); scale bar= 200 µm. D—prosoplectenchymatic parathecium with irregularly orientated hyphae (Wilk 9380, LPB); scale bar= 100 µm.

Iñao, close to Ticucha, 19°37′26″S 63°50′55″W, alt. 1040 m, disturbed Sub-Andean Boliviano-Tucumano forests with Acacia, 18 July 2015, Flakus 26310 (KRAM, LPB). **ARGENTINA. Prov. Corrientes, Dept. Concepción**, 11 km N de Santa Rosa, 24 Jan. 1974, M.M. Arbo 504 (S).

***Caloplaca letrouitioides*—AUSTRALIA. Victoria, Gippsland Plain,** Gunnamatta Beach, 1976, R.B. Filson 15871 (MEL, **holotype**).

Comments—The combination *Caloplaca crocina* was suggested by Marcelli in the original version (dated 2002, unpublished) of the Brazil lichen checklist, as "*Caloplaca crocinum* [sic] (Krempelhuber) não comb. [i.e., not combined]". After checking subsequent Marcelli publications and making wide enquiries amongst lichenologists in South America and internationally, we have found no evidence that this name was ever validated; Feuerer (2015) cited the species only as *Blastenia crocina*.

Caloplaca crocina is diagnosed by its continuous to rimose creamy thallus, zeorine apothecia with orange discs, and hourglass-shaped ascospores [sensu

Navarro-Rosinés et al. 2000; spore walls 1–2(–3) µm thick]. The apothecial thalline margin is usually much reduced and its fragments are visible at the base of apothecia. In contrast, the proper margin is thick and swollen, similar to that of the biatorine apothecia in *Letrouitia*. *Caloplaca crocina* is superficially very similar to *Letrouitia*, which differs in its multi-septate to muriform spores. Two other *Caloplaca* species resemble *Letrouitia* spp.: *Caloplaca letrouitioides* S.Y. Kondr. et al. and *C. kiewkaensis* Yakovczenko et al. (Kondratyuk et al. 2011). *Caloplaca letrouitioides* differs from *C. crocina* in having truly biatorine apothecia with a more swollen proper margin that partially overlaps the disc, apothecial discs at first distinctly concave, and thin-walled ascospores. *Caloplaca kiewkaensis* is distinguished by its smaller ellipsoid to almost spherical spores, a parathecium consisting of radiating hyphae, and a different Far East Russian distribution. See Table 1 for a comparison of *C. crocina* with *Caloplaca* species characterized by thick-walled ascospores—*C. calcitrapa* Nav.-Ros. et al., *C. cupulata* Poelt & Hinter., *C. dichroa* Arup, *C. haematommona* Elix & S.Y. Kondr., *C. lobulascens* Poelt & Hinter., *C. pollinii* (A. Massal.) Jatta, *C. rheinigera* Elix & S.Y. Kondr., and *C. yuennana* (Zahlbr.) Poelt & Hinter.

In Bolivia, *C. crocina* occurs mainly on tree bark (rarely on wood) in savannas and relatively dry woodlands (e.g., Chaqueño and Chiquitano forests, Tucumano-Boliviano forest, and submontane anthropogenic woodlands) at altitudes between 190 and 1635 m. The species is very frequent in these areas. *Caloplaca crocina* occurs in subtropical and tropical regions of Argentina, Brazil, Paraguay (Malme 1926) and Uruguay (Osorio 1972). This is the first report from Bolivia.

Other species

Caloplaca baueri (Müll. Arg.) Zahlbr., Denkschr. Kaiserl. Akad. Wiss., Wien, Math.-Naturwiss. Kl. 83: 192. 1909.

Specimens examined: ***Caloplaca baueri***—**BOLIVIA. Dept. Beni, Prov. Itenez**, Cerro de Oricore by Río San Martín, 13°19′55″S 63°30′37″W, alt. 240 m, dry forest overgrow granitic hill (an inselberg), 19 Aug. 2008, Flakus 12680/1 & Rodriguez (KRAM, LPB). **Dept. La Paz, Prov. Muñecas**, Camata village, NE slope, 15°14′34″S 68°45′06″W, alt. 2020 m, open place, on a stone fence, 15 May 2006, Wilk 4436 (KRAM).

Caloplaca subunicolor—**BRAZIL. Matto Grosso**, pr. Cuyaba, 6 June 1894, G. Malme (LD 1036560).

Caloplaca baueri belongs to the *C. cinnabarina* group (Wetmore & Kärnefelt 1999). It is characterized by its continuous to rimose or only slightly areolate, smooth and glossy, greenish-yellow to yellow-orange thallus that gradually thins at the margin, with a minimal yellow prothallus. Apothecia are frequent. The

TABLE 1. Comparison of *Caloplaca crocina* and other *Caloplaca* spp. that produce thick-walled ascospores with ± reduced lumens (including typical hourglass-shaped spores)

CHARACTERS	*crocina*	*calcitrapa*	*cupulata*	*dichroa*
SPORES (µm)	15–24 × 8–11	11.5–15 × 6–8	16–21 × 8–13	12–16.5 × 6–8.5
SEPTUM (µm)	4–9	3–6	4–7	2.5–4
WALL (µm)	1.5–2.5	1–2	Unknown	1–2
APOTHECIA	Zeorine	Zeorine	Zeorine	Zeorine
DISC	Brownish orange	Orange	Brownish to red-orange	Orange
PROP MARGIN	Orange	Orange	Orange	Orange
K RXN	+, purple	+, purple	Unknown	+, purple
THAL MARGIN	+, ± fragmented near base	+	+	+
EXCIPLE	Parathecium, thick	Parathecium	Parathecium	Parathecium
PLECTENCHYMA	Prospo-	——	Para-	——
HYPH. ORIENT	Irregular	Radiating	——	Radiating thick-w
AMPHITHECIUM	Weakly dev.	+	+	+
ALGAE	Distinct groups	Abundant	Abundant	Abundant
THALLUS	Creamy	Yellow-orange,	White	Yellow to orange
K RXN	K–	K+ purple	K–	K+ purple
MORPHOLOGY	Continuous to rimose	Granulose-areolate	Granulose-areolate to squamulose	Areolate, with blastidia
SUBSTRATE	Bark (rarely wood)	Calcareous rocks	Bark, wood	Calcareous rocks
HABITAT	Dry woodland	Medit. vegetation	Arid forests	Sun-exposed sites
DISTRIBUTION	SAm—Argentina Bolivia Brazil Paraguay Uruguay	EUR—France Spain Italy; AFR—Algeria Morocco	Nepal	Europe
REFERENCES	Malme (1926); this paper	Navarro-Rosinés et al. (2000)	Poelt & Hinteregger (1993)	Arup (2006)

(concluded on p. 132)

Table 1, concluded

Characters	*haematommona*	*lobulascens*	*pollinii*	*rheinigera*	*yuennana*
Spores (µm)	10–13 × 5–7	9–14 × 5–7	12.5–17 × 7–10	15–19 × 7–9	21–30 × 11–14
septum(µm)	4–6	2–5	4–7	3–7	3–5
wall (µm)	c. 1	Unknown	c. 1	1–2	unknown
Apothecia	Biatorine (occ. zeorine)	Zeorine	Biatorine	Biatorine	Biatorine (occ. zeorine)
disc	yOr to brOr	Orange-brown	Brwn/rust/blk	Rust-brown	Olive-black
prop margin	yOr to brOr	Orange-brown	Brwn/rust/blk	Rust-brown	Red-brown
K rxn	+ purple	Unknown	+ rViol > prp	Red-violet	Unknown
thal margin	Very thin/ —	+/evanescent	—	—	—/+ at base
Exciple	Parathecium	Parathecium	Parathecium	Parathecium	Parathecium
plectench.	Para-	Para-	——	——	——
hyph. orient	Conglut. matrix	——	Radiating, thick-walled	Radiating, conglut.	Radiating
amphithec.	+/–	+	–	–	– (rarely +)
algae	——	Abundant	——	——	(few below)
Thallus	Whitish	Whitish	Grey	Grey	brW
K rxn	Unknown	Unknown	K–	Unknown	Unknown
Morph.	Continuous, very thin to almost absent	Rimose-areolate, + phyllidia	Continuous to areolate	Continuous	Continuous to areolate
Substrate	Bark on shrub twigs	Siliceous rock	Bark, wood	Bark on twigs	*Picea* bark
Habitat	Dry woodland	Warm temperate	——	Dry woodland	Temperate mixed forest
Distribution	Australia	Nepal	Eur NAm Asia	Australia	China
References	Kondratyuk et al. 2007	Poelt & Hinteregger 1993	Wetmore 1994	Kondratyuk et al. 2007	Poelt & Hinteregger (1993)

species resembles the European *C. ochracea* (Schaer.) Flagey, which produces spores with four locules, non-corticate thallus, and a habit on calcareous rocks. *Caloplaca baueri* is very similar to *C. subunicolor* (Nyl.) Zahlbr., which differs in having a more areolate, darker orange thallus and lack of prothallus; the geographical distribution of both species is also quite different, as *C. subunicolor* is centered in Africa (Wetmore & Kärnefelt 1999). Moreover, *C. baueri* may also be confused with *C. cinnabarina*, which has a distinctly areolate bright orange thallus with elongated areoles at the thallus margin. Wetmore & Kärnefelt (1999) and Wetmore (2007) provide a detailed description of *C. baueri.*

The species occurs in Bolivia on silicate outcrops in dry forest, surrounded by the humid Amazonian zone. *Caloplaca baueri* is known from Central and South America, and the Caribbean region; in South America Wetmore & Kärnefelt (1999) have reported *C. baueri;* this is the first report from Bolivia.

Caloplaca cinnabarina (Ach.) Zahlbr., Nat. Pflanzenfam., 1(1*): 228. 1908.

Exsiccati examined—Wetmore, Telos. Exsicc. 28, 29 (KRAM). Weber, Lich. Exs. 446 (as *Caloplaca subnitida*) (KRAM).

Specimens examined—**BOLIVIA. Dept. La Paz, Prov. Franz Tamayo**, Parque National y Área Natural de Manejo Integrado Madidi, along the way from Mojos to Insensio, 14°33′22″S 68°52′27″W, alt. 1635–1960 m, sunny, dry area with sparse trees and shrubs, 1 Nov. 2007, Wilk 9545 (KRAM, LPB). **Dept. Santa Cruz, Prov. Chiquitos**, near Santa Cruz de la Vieja, mirador a San José de Chiquitos village, 17°52′21″S 60°45′41″W, alt. 501 m, Cerrado de la Chiquitanía (wooded savannas), 5 Dec. 2010, Flakus 19376, Quisbert & Wolski (KRAM, LPB). **AUSTRALIA. New South Wales**, 'Bush Bottoms', NE of Goulburn, 18 Oct. 1987, H. Streimann 38875 (B).

Caloplaca cinnabarina is characterized by its bright orange areolate thallus with the areoles slightly elongated at the thallus margin. The apothecia are more or less immersed in and concolorous with the thallus. See Wetmore & Kärnefelt (1999), Wetmore (2007) and Joshi et al. (2011) for detailed descriptions. To distinguish *C. cinnabarina* from *C. baueri* see remarks above under that species. The recently described Australian species, *C. brownlieae* S.Y. Kondr. et al., differs from *C. cinnabarina* in having a dull pink to dull brownish-orange thallus and wider paraphyses tips, ascospores, and spore septa. According to Lumbsch et al. (2011), *C. brownlieae* also differs chemically in having ovoic and lecanoric acids and a high concentration of gyrophoric acid. *Caloplaca rubelliana* (Ach.) Lojka, a primarily European species, is very similar to *C. cinnabarina*, but is distinguished by its greyish-orange or brownish-orange thallus that thins at the margin and scarlet red apothecia; moreover, it does not form elongated areoles at the thallus margin (Wetmore & Kärnefelt 1999).

Caloplaca cinnabarina occurs in Bolivia on silicate rocks in dry woodland areas. This rather well known species, widespread in subtropical and tropical

regions, has been reported from North and Central America (Wetmore & Kärnefelt 1999), Africa (Kärnefelt 1988), Australia (Kärnefelt 2003), New Zealand (Galloway 2007), and Asia (Joshi et. al. 2010). In South America it is known from Argentina (Calvelo & Liberatore 2002), Uruguay (Osorio 1983) and Venezuela (Aptroot 2015). This is the first report from Bolivia.

Caloplaca darbishirei (C.W. Dodge & G.E. Baker) Cretz., Bul. Grăd. Bot. Univ. Cluj 21: 140. 1941.

Specimen examined—**BOLIVIA. Dept. Potosi, Prov. Nor Lipez**, Pinturas Rupestres near Mallku Villamar village, 21°46′20″S 67°29′05″W, alt. 4038 m, high Andean area, 6 Dec. 2009, Flakus 14802 (LPB).

The species is characterized by its orange-yellow, squamulose, and sorediate thallus. The squamules are peltate with incised margins, and the soralia develop on the underside of the squamule margins. Soredia are concolorous with the thallus. The closely related *Caloplaca soropelta* (E.S. Hansen et al.) Søchting differs in its less incised squamule margins and golden yellowish soredia distinctly lighter than the thallus. For more information about both species see Søchting & Castello (2012).

Caloplaca darbishirei occurs in Bolivia on silicate rock together with *C. texana* in high montane regions. It is known from Antarctica and the southernmost part of Argentina in South America (Søchting & Castello 2012). This is the first report of *C. darbishirei* from Bolivia.

Caloplaca ochraceofulva (Müll. Arg.) Jatta, Nouv. Giorn. Bot. Ital., n.s. 17: 194. 1910.

Exsiccati examined—Almborn, Lich. africani 69 (as *C. subnitida*) (LD).

Specimens examined: ***Caloplaca ochraceofulva*—BOLIVIA. Dept. La Paz, Prov. Bautista Saavedra**, Charazani village, alt. c. 3000 m, by the road, open place, 13 May 2006, Wilk 4144 (KRAM). **Dept. Cochabamba, Prov. Quillacollo**, area of Inkarraya-Sipesipe, semidesert Inter-Andean Valleys, 17°29′25″S 66°22′09″W, alt. 3146 m, rocky and shrubby slope, sunny place, E exp., 17 Dec. 2004, Wilk 3277 (KRAM, LPB). **ARGENTINA. Prov. Jujuy**, Santa Barbara, alt. 1300 m, 9 July 1901, Rob. E. Fries 50 (LD).

***Caloplaca isidiosa*—BRAZIL. Bahia**, zwischen Feira de Santana und Milagres, alt. 200 m, an bodennahen Granitblöcken in einer Caatinga, 21 July 1980, K. Kalb [Lichenes neotropici no. 207] (B).

Caloplaca ochraceofulva is characterized by its placodioid thallus with moderately long and flat lobes. The central thallus is rimose-areolate, with numerous isidia produced on the margins of rather thick subsquamulose areoles. The species usually occurs in the sterile form. For more information see Malme (1926, as *C. subnitida*), Kärnefelt (1990) and Wetmore & Kärnefelt (1998). The similar

C. texana differs in having the central thallus densely covered by phyllidia. *Caloplaca ochraceofulva* may also be confused with *C. isidiosa* (Vain.) Zahlbr., which is distinguished by its coralloid or simple elongate isidia that densely cover the central thallus and frequent apothecia (Kärnefelt 1988, 1990).

Caloplaca ochraceofulva occurs in Bolivia on silicate or slightly calcareous rocks in high Andean regions at an altitude of c. 3000 m. It has an African-South American distribution pattern (Kärnefelt 1988) and has been recorded from Argentina (Malme 1926), Brazil (Osorio & Fleig 1990), Peru (Ramos 2014), and southern and eastern Africa, including one locality on the Arabian Peninsula (Kärnefelt 1990). This is the first report from Bolivia.

Caloplaca squamosa (B. de Lesd.) Zahlbr., Cat. Lich. Univ. 10: 629. 1940.

Exsiccati examined—Weber, Lich. Exs. Colo. 414 (as *Caloplaca modesta*) (KRAM).

Specimen examined—**BOLIVIA. Dept. Santa Cruz, Prov. Caballero**, area of Siberia village, 17°49′38″S 64°45′14″W, alt. 3480 m, the field on hill above road (on the fringes of the Yungas cloud forest), open, foggy and windy area, on sandstone, 15 Dec. 2004, Wilk 3049 (KRAM, LPB), 3073, 3125 (KRAM).

Caloplaca squamosa and *C. subsoluta* (discussed below) belong to the *C. squamosa* group, which is characterized by a more or less squamulose thallus, orange apothecia, and a paraplectenchymatous apothecial proper margin (Wetmore 2003). Both *C. squamosa* and *C. subsoluta* are highly variable and more study is needed to refine their taxonomy. Typical *C. subsoluta* differs from *C. squamosa* by its areolate to subsquamulose thallus lacking short lobes and smaller apothecia lacking distinct thalline margins. For more information on both species see Wetmore (2003, 2007).

Caloplaca squamosa occurs in Bolivia on silicate rocks in open anthropogenic areas close to Yungas cloud forest at an altitude of c. 3000 m. Its centre of distribution lies in southwestern North America (Wetmore 2003, 2007). In South America it is known from the Galapagos Islands (Bungartz et al. 2013). This is the first report from Bolivia.

Caloplaca subsoluta (Nyl.) Zahlbr. s. lat., Cat. Lich. Univ. 7: 185. 1931.

Exsiccati examined—Wetmore, Telos. Exsicc. 71, 72, 73, 92 (KRAM). Suza, Lich. Bohemoslov. 206 (as *C. irrubescens*) (KRAM).

Specimens examined—**BOLIVIA. Dept. Santa Cruz, Prov. Caballero**, area of Siberia village, 17°49′38″S 64°45′14″W, alt. 3480 m, a field on hill above road (on the fringes of the Yungas cloud forest), open, foggy and windy area, 15 Dec. 2004, Wilk 3134 (KRAM, LPB). **Dept. Cochabamba, Prov. Quillacollo**, area of Inkarraya-Sipesipe, 17°29′25″S 66°22′09″W, alt. 3146 m, semidesert Inter-Andean Valleys, rocky and shrubby slope, sunny place, E exp., 17 Dec. 2004, Wilk 3228 (LPB). **PERU. Arequipa, Prov. Caylloma**, Valle del Colca valley, near Soccoro village, 15°38′32″S 71°43′22″W, alt. 3349 m, open semi-desert montane area, 3 July 2006, Flakus 9409 & Cykowska (KRAM).

Wetmore (2003) also treats *Caloplaca subsoluta* in the *C. squamosa* group. The species, together with *C. americana* (Malme) Zahlbr., *C. irrubescens* (Arnold) Zahlbr., and *C. modesta* (Zahlbr.) Fink form a group of very similar species, but the taxonomic affiliation is unclear. The interpretation of these species varies according to author; for example, Wetmore (2003) treats *C. americana*, *C. irrubescens*, and *C. modesta* as synonyms of *C. subsoluta*. For comparison with *C. squamosa*, see comments above under that species.

Caloplaca subsoluta occurs in Bolivia on sandstone rocks in open anthropogenic areas at altitudes above 3000 m. The species is distributed worldwide and has been reported from Europe (e.g. Hafellner & Türk 2001), North America (Wetmore 2003), Asia (e.g. Joshi et al. 2010), and Africa (e.g. Fryday 2015). In South America it has been recorded from Argentina, Brazil (Malme 1926, as *Callopisma americanum*), Peru (Ramos 2014), Uruguay (Osorio 1983, as *Caloplaca americana*), Venezuela (Aptroot 2015), and the Galapagos Islands (Bungartz et al. 2013). This is the first report from Bolivia.

Caloplaca texana Wetmore & Kärnefelt. Bryologist 101(2): 247. 1998.

Exsiccati examined—Wetmore, Telos. Exsicc. 8, 74, 75 (KRAM). Nash, Lich. Exs. 256 (KRAM).

Specimens examined—**BOLIVIA. Dept. Potosi, Prov. Nor Lipez**, Pinturas Rupestres near Mallku Villamar village, 21°46′20″S 67°29′05″W, alt. 4038 m, high Andean area, 6 Dec. 2009, Flakus 14802/1 (KRAM), Flakus 14812 & Rodriguez (LPB). **PERU. Arequipa, Prov. Caylloma**, Valle del Colca valley, near Soccoro village, 15°38′32″S 71°43′22″W, alt. 3349 m, open semi-desert montane area, 3 July 2006, Flakus 9404, 9408, 9411, 9414, 9415 & Cykowska (KRAM).

Caloplaca texana is a distinctive species distinguished by its thick placodioid thallus that produces moderately long flat marginal lobes ±loosely attached to the substratum. The central thallus is areolate with numerous phyllidia. The species usually occurs in the sterile form. The similar *C. ochraceofulva* is distinguished by its globose isidia on the squamulose areole margins. *Caloplaca texana* may be confused with *C. appressa* Wetmore & Kärnefelt, which differs in its marginal lobes tightly appressed to the rock, frequent reddish apothecia, and lack of vegetative diaspores (Wetmore & Kärnefelt 1998).

Caloplaca texana occurs in Bolivia on silicate rocks in high montane areas at altitudes above 4000 m. It was originally described from North America, and these Bolivian and Peruvian records represent the first reports from the Southern Hemisphere.

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Notes on rust fungi in China 3. *Puccinia adenocauli* comb. nov. and its life cycle and new host

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Abstract—The heteroecious and macrocyclic life cycle of *Aecidium adenocauli* was proved with field observations and inoculation experiments in China. Its spermogonial and aecial stages were produced on *Adenocaulon himalaicum,* and uredinial and telial stages were produced on *Carex onoei,* which is a new host for this rust fungus. Uredinial and telial stages (teleomorph) of this rust fungus were formerly described as *Puccinia carici-adenocauli* from Japan. However, the current International Code of Nomenclature (ICN (Melbourne)) has abolished the distinction between "anamorph" names and "teleomorph" names, and we propose the new combination *Puccinia adenocauli*, based on *Ae. adenocauli* 1913, which now has priority over *P. carici-adenocauli* 1999. Its neotype and epitype are also designated.

Key words—*Asteraceae*, *Cyperaceae, Pucciniomycetes,* taxonomy, *Uredinales*

Introduction

Aecidium adenocauli has been reported to produce spermogonial and aecial stages (anamorph) on *Adenocaulon himalaicum* Edgew. (*Asteraceae*) from Japan, Korea, Russian Far East, and China (Sydow & Sydow 1913, Ito 1950, Hiratsuka 1963, Azbukina 1974, Zhuang 1990, Hiratsuka et al. 1992). However, Kakishima et al. (1999) showed with inoculation experiments that its uredinial and telial stages (teleomorph) were produced on *Carex hakonensis* Franch. & Sav. and *C. uda* Maxim. (*Cyperaceae*) in Japan and described this teleomorph as a new species, *Puccinia carici-adenocauli.* Azbukina (2005) also reported

this species on *Ad. himalaicum* and *C. uda* from the Russian Far East based on morphological observations.

During investigations of rust fungi in Jilin Province, northeast China, we found the aecial stage of a rust fungus on *Ad. himalaicum*. This rust fungus was morphologically similar to *Ae. adenocauli,* but its uredinial and telial stages had not been clarified in China. Therefore, we carried out field survey and inoculation experiments to determine life cycle of this rust fungus. We report here the results of inoculation experiments with this rust fungus and its morphology based on specimens collected in China. We also discuss the taxonomic treatment of this fungus according to the current International Code of Nomenclature for algae, fungi, and plants (ICN (Melbourne); McNeill et al. 2012) and propose the new combination *Puccinia adenocauli*.

Materials & methods

Field survey and inoculation experiments

An aecial stage on *Ad. himalaicum* was found in the forest floor at Hongyegu, Jiaohe, Jilin, Jilin Province, China (43°42′13″N 127°04′18″E, alt. 537m) in June 2015 during a survey of rust fungi. This forest mainly consists of species of *Betula, Populus*, and *Quercus* and is a typical deciduous broad-leaved forest in this area. Uredinial and telial stages of this rust fungus were suspected to occur on species of *Carex* because a heteroecious species, *P. carici-adenocauli,* alternating between species of *Adenocaulon* and *Carex,* had been reported from Japan (Kakishima et al. 1999) and the Russian Far East (Azbukina 2005). Therefore, we looked for rust fungi on *Carex* species in the area where the aecial stage on *Ad. himalaicum* had occurred and collected telial stages on *C. onoei* Franch. & Sav. and *C. ussuriensis* Kom. in September 2015 and May 2016. These telial stages were morphologically identified as belonging to *Puccinia* and were used for inoculations.

Inoculation experiments were carried out during May–June 2016 at Jilin Agricultural University, Changchun, Jilin Province. For basidiospore inoculations the leaves with telia collected on 13 May 2016 (Fig. 2B) were kept in water for 1–2 hours to induce teliospore germination. These leaves were cut into small pieces (ca. 5 mm^2) and placed on healthy leaves of *Ad. himalaicum,* which was transplanted from the same forest cited above and maintained in plastic pots at Jilin Agricultural University. No natural infection of the plant from fields was confirmed before inoculations. The inoculated plants were kept in a moist atmosphere in a plastic box in darkness at 15–20°C for three days and then transferred to a place near windows at 15–20°C for observations.

Aeciospores produced on *Ad. himalaicum* by basidiospore inoculations were then used as inocula. Aeciospores were dusted onto wet filter papers (about 3 mm^2) and these papers were then placed on young healthy leaves of *C. onoei* planted in plastic pots. The plants were kept in a plastic box under the same conditions as basidiospore inoculations, later transferred to a place near windows.

Morphological observations

Specimens collected in the field or obtained from inoculations were used for morphological observations. Specimens of *Puccinia carici-adenocauli* including the type specimen (TSH-R1693) deposited in the Mycological Herbarium of the Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Japan (TSH), were borrowed for comparative morphology.

Light microscopy (LM) was used to examine morphological characters including the size and shape of sori and spores. Spores or thin-sections of sori from specimens were mounted in a drop of lactophenol solution on glass slides for LM. Approximately 50 spores from each specimen were randomly chosen and the length, width, and wall thickness of spores were measured using Leica LAS X software attached to a Leica DM2000 microscope (Leica, Germany).

The surface features of spores were examined by scanning electron microscopy (SEM). For SEM, sori and spores obtained from dry specimens were attached to specimen holders by double-sided adhesive tape and coated with platinum-palladium using a Hitachi MC1000 Ion Sputter Coater and examined with a Hitachi SU8010 SEM operated at 5–7 kV.

Dry specimens used in the experiments were deposited in the Herbarium of Mycology, Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi, Jilin Agricultural University, China (HMJAU).

Results & discussion

Life cycle

Seven to ten days after inoculations with basidiospores from teliospores on *C. onoei,* small yellow spots of spermogonia appeared on the upper leaf surface of *Ad. himalaicum* (Fig. 1A). About 7 days later, cupulate aecia with aeciospores were produced on the lower leaf surface of the same leaves (Fig. 1B). No infection was observed on *Ad. himalaicum* in the inoculations with teliospores from *C. ussuriensis;* this suggests that the *C. ussuriensis* rust is probably a different species from the rust on *C. onoei*. About 12 days after inoculations with aeciospores from *Ad. himalaicum,* pale yellow spots appeared on *C. onoei* and then powdery uredinia were produced (Figs 2C, 2E). The inoculation results confirmed that the rust fungus producing spermogonial and aecial stages on *Ad. himalaicum* was conspecific with the rust fungus producing uredinial and telial stages on *C. onoei.*

Morphology & taxonomy

Morphological observations using LM and SEM of specimens obtained from the field and inoculations showed that spermogonial and aecial stages on *Ad. himalaicum* and uredinial and telial stages on *C. onoei* were identical with the morphology of *P. carici-adenocauli* (anamorph: *Ae. adenocauli*) described by Kakishima et al. (1999). Their identity was also confirmed by comparative

morphology with specimens from TSH. However, according to McNeill et al. (2012: Art. 59.1), since 1 Jan. 2013, the earliest legitimate holomorphic name of this species is *Ae. adenocauli*, and *P. carici-adenocauli* is a later synonym (Kakishima et al. 1999). Therefore, we propose a new name based on the earlier name. Sydow & Sydow (1913) described *Ae. adenocauli* based on the specimen on "*Ad. bicolor*" [a misdetermination of *Ad. himalaicum*] collected at Kuzumaki-machi, Iwate Prefecture, Japan on 6 July 1907. This specimen was not found in any herbarium, including B (Berlin, Germany), and it is suspected that the specimen was lost during World War II. Therefore, we designate a neotype specimen on *Ad. himalaicum* for this species and also an epitype specimen on *Carex onoei*. We also provide a description based on specimens obtained in China.

Puccinia adenocauli (Syd. & P. Syd.) Jing X. Ji & Kakish., **comb. nov.** Figs 1–3

MycoBank MB817618

≡ *Aecidium adenocauli* Syd. & P. Syd., Ann. Mycol. 11: 111, 1913.

Type: Stages 0, I on *Adenocaulon himalaicum,* Japan, Yamanashi Prefecture, Yamanakako-mura, 25 May 1983, leg. M. Kakishima (**Neotype designated here**, TSH-R1689). Stage III on *Carex onoei*, China, Jilin Province, Jilin, Jiaohe, 13 May 2016, leg. J.X. Ji & M. Kakishima (**Epitype designated here**, HMJAU8275).

= *Puccinia carici-adenocauli* Kakish., Yokoi & Y. Harada, Mycoscience 40: 506, 1999.

Spermogonia subepidermal (type 4 of Cummins & Hiratsuka 2003). Aecia subepidermal, erumpent, *Aecidium*-type with peridia and catenulate spores. Aeciospores catenulate, globose, obovoid or sometimes angular, 13–18 × 10–15 µm (av. 15 × 12 µm), walls hyaline, 0.6 µm thick, verrucose with large granules. Uredinia subepidermal, pale yellow, erumpent, without paraphyses. Urediniospores pedicellate, obovoid or ellipsoid, 21–27 × 16–22 µm (av. 23 × 20 µm), walls hyaline, echinulate, 1.6–1.2 µm (av. 0.9 µm), germ pores obscure. Telia subepidermal, erumpent, dark brown to black. Teliospores 2-celled by transverse septum, borne singly on pedicels, clavate to oblong with round to obtuse apex and attenuate toward bases, constricted at septum, 30–47 × 15–26 µm (av. 40 × 20 µm), walls dark brown, smooth, 0.6–2.0 µm (av. 1.2 µm) thick, thickened at apex.

Specimens examined — **China, Jilin Province:** Stages 0, I on *Adenocaulon himalaicum*, (Jilin, 24 June 2015, HMJAU8205, cultured at Jilin Agricultural Univ., Changchun, 11 June 2016, HMJAU8277, cultured at Jilin Agricultural Univ., Changchun, 24 June 2016, HMJAU8278, Tonghua, 8 June 2016, HMJAU8276); Stage II on *Carex onoei*, 2 July 2016, cultured at Jilin Agricultural Univ. (HMJAU8274); Stage III on *C. onoei*,13 May 2016, Jilin (HMJAU8275).

Hosts & distribution — Stages 0, I on *Adenocaulon himalaicum:* China (Zhuang 1990), Korea (Ito 1950, Hiratsuka 1963, Hiratsuka et al. 1992, Kakishima et al. 1999), Russian Far East (Azbukina 1974, 2005, Kakishima et al. 1999), Japan (Ito 1950,

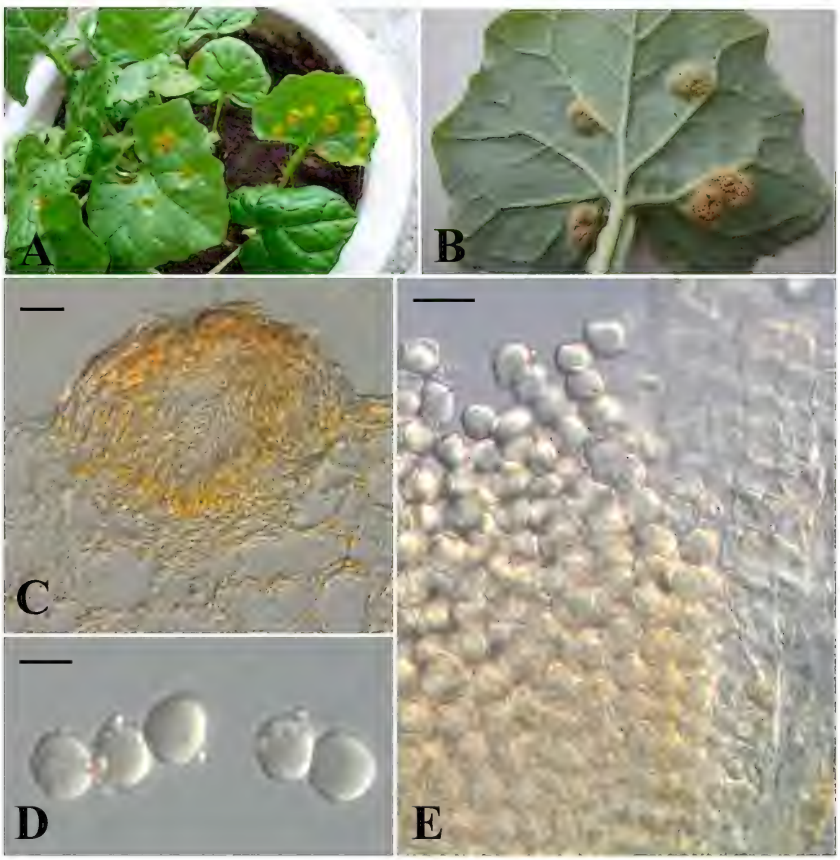

Fig. 1. *Puccinia adenocauli* on *Adenocaulon himalaicum*: spermogonial and aecial stages. A. Spermogonia on upper leaf surfaces produced by basidiospore inoculation. B. Aecia on lower leaf surface produced by basidiospore inoculation. C. Vertical section of a spermogonium. D. Aeciospores with large granules. E. Catenulate aeciospores surrounded by peridium in a vertical section of an aecium. Scale bars: C, D = 15 μm; E = 20 μm.

Hiratsuka et al. 1992, Kakishima et al. 1999). Stages II, III on *Carex hakonensis:* Japan (Kakishima et al. 1999). II, III on *C. uda:* Russian Far East (Azbukina 2005), Japan (Kakishima et al. 1999). Stages II, III on *C. onoei:* China.

The frequent occurrence of aecial stage on *Ad. himalaicum* indicates that this rust fungus is widely distributed in East Asia, although its uredinial and telial stages on *Carex* species have been rarely reported. Collection of this rust fungus on *Carex* is difficult because *Carex* species are short and their leaves are very

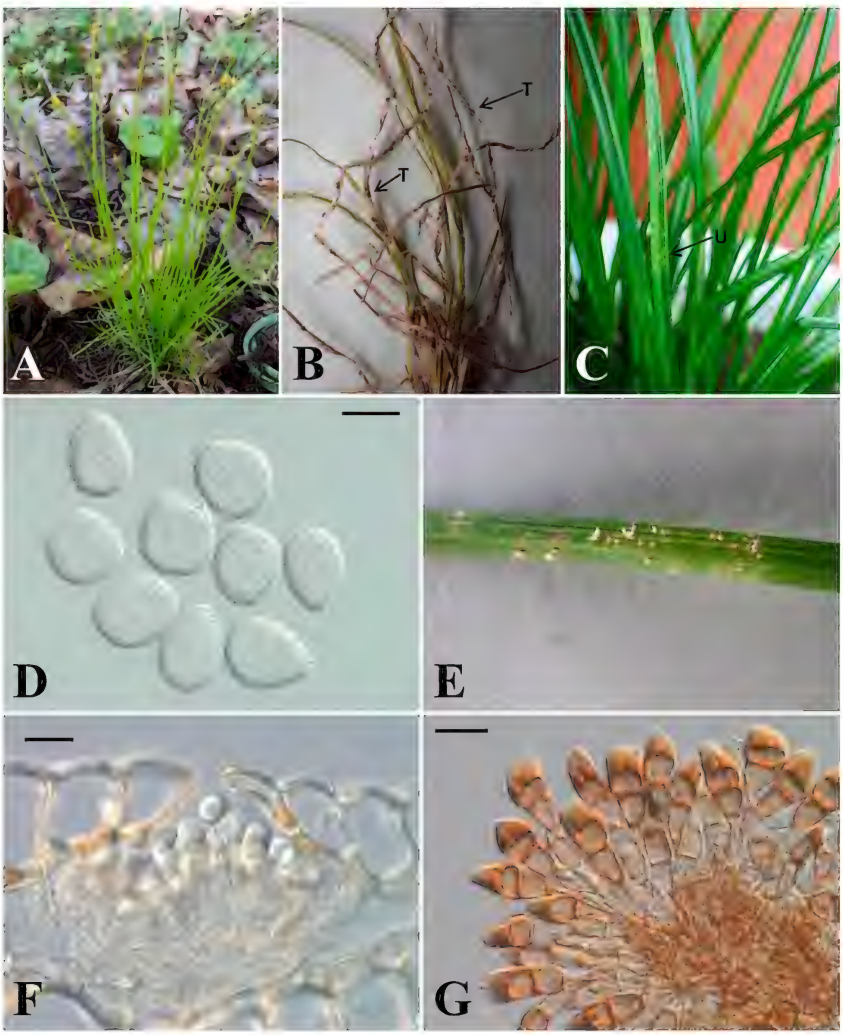

Fig. 2. *Puccinia adenocauli* on Carex *onoei*: uredinial and telial stages. A. *Carex onoei* with flowers taken on forest floor at Jilin, Jilin Province in May 2016. B. Dark brown telia (T) on the dead leaves. C. Uredinia (U) on the leaf surface produced by aeciospore inoculation. D. Echinulate urediniospores obtained by aeciospore inoculation. E. Pale yellow uredinia on lower leaf surface produced by aeciospore inoculation. F. Vertical section of a uredinium. G. Teliospores. Scale bars: D = 20 μm; F = 50 μm; G = 30 μm.

thin. Our field observations and inoculations have shown, for the first time, that *C. onoei* serves as uredinial and telial host of this rust fungus in China.

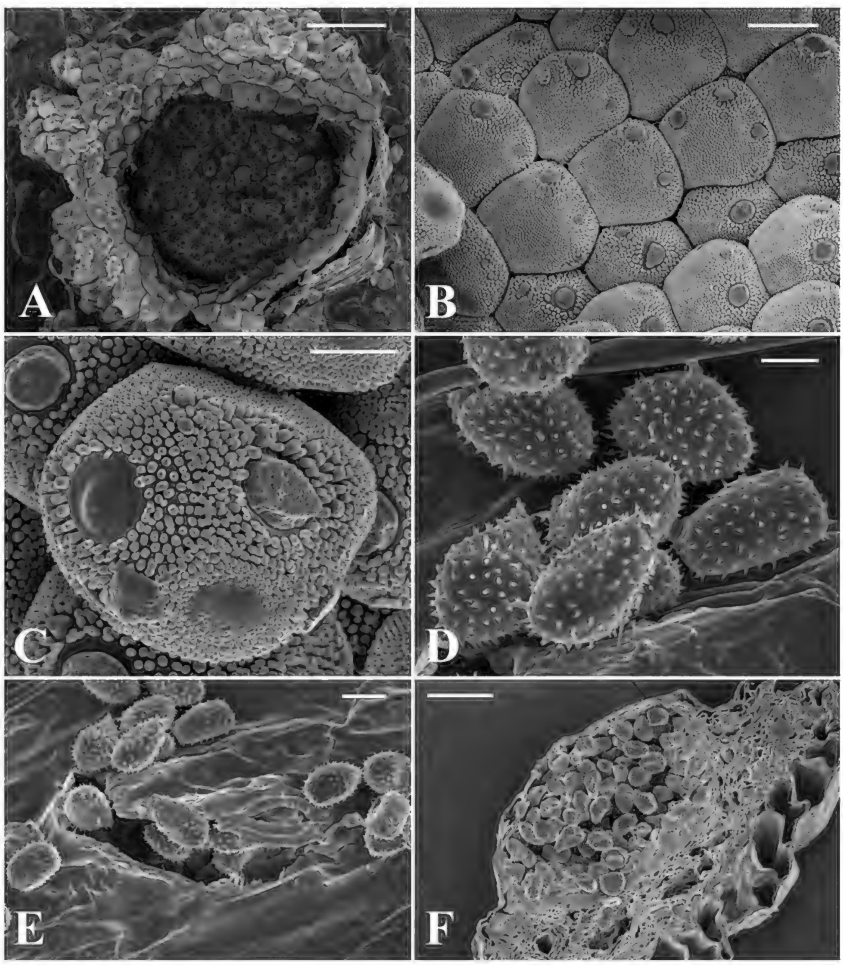

Fig. 3. *Puccinia adenocauli* observed with SEM. A. Aecium. B. Catenulate aeciospores in an aecium on *Adenocaulon himalaicum*. C. Verrucose aeciospore with large granules. D. Echinulate urediniospores. E. Uredinium produced on the leaf surface of *Carex onoei*. F. Vertical section of a uredinium. Scale bars: A = 40 µm; B = 10 µm; C = 5 µm, D = 15 µm; E = 20 µm; F = 50 µm.

Carex onoei also represents a new host for this rust fungus. Two other *Carex* spp., which are uredinial and telial hosts of this rust, have been reported from Japan and the Russian Far East (Kakishima et al. 1999, Azbukina 2005). These three *Carex* species (*C. hakonensis*, *C. onoei*, *C. uda*) belong to *Carex* sect. *Rarae* and it is, therefore, probable/possible that this rust fungus may be host specific to this section of *Carex* for its survival.

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Colletotrichum fioriniae comb. & stat. nov., resolving a nomenclatural muddle

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Abstract—The widely used name "*Colletotrichum fioriniae*", published in 2009, is invalid; it is neither a valid new combination for "*Colletotrichum acutatum* var. *fioriniae*" (itself invalid) nor a valid new name, because no Latin diagnosis or description was presented or referenced. With the abolition of "dual nomenclature" (the separate nomenclatures of teleomorphs and anamorphs) under the current International Code of Nomenclature, it is now possible to publish *Colletotrichum fioriniae* as a new combination based on *Glomerella acutata* var. *fioriniae*, the only validly published name for this taxon.

Key words—*Glomerellaceae*, *Glomerella fioriniae*, *Colletotrichum acutatum* group A3, *Colletotrichum acutatum* group C

Introduction

Glomerella acutata var. *fioriniae* was proposed with an accompanying Latin diagnosis by Marcelino et al. (2008), together with an asexual morph, "*Colletotrichum acutatum* var. *fioriniae*". In 2008, the then current International Code of Botanical Nomenclature (ICBN (Vienna); McNeill et al. 2006: Art. 59.1) permitted the simultaneous publication of separate names for the asexual and sexual morphs of a single taxon. However, the lack of a Latin diagnosis or description means that Marcelino et al.'s (2008) *Colletotrichum* name is invalid (McNeill et al. 2012: Art 39.1).

Within *Colletotrichum acutatum* sensu lato, Shivas & Tan (2009) identified a separate clade ("formerly *C. acutatum* group C [of Lardner et al. 1999] or A3 [of Sreenivasaprasad & Talhinhas 2005]"), for which they proposed that the two varietal names of Marcelino et al. (2008) should be raised to species rank

as "*C. fioriniae*" (anamorph) and "*G. fioriniae*" (teleomorph). Unfortunately, both of these names are invalid: the proposal of "*Colletotrichum fioriniae*" cites (with a full and direct reference) the invalid name "*C. acutatum* var. *fioriniae*" as its "basionym", and fails to supply the required Latin diagnosis or description; the proposal of "*Glomerella fioriniae*" fails to provide the required full and direct reference to its basionym (McNeill et al. 2012: Art 41.5) and indeed does not clearly indicate the name of its basionym (although in subsequent discussion, *G. acutata* var. *fioriniae* is mentioned in passing).

This leaves *Glomerella acutata* var. *fioriniae* as the only valid name available for the taxon.

The species has been widely treated under the invalid name "*Colletotrichum fioriniae*" in recent taxonomic and phytopathological literature (e.g., Baroncelli et al. 2015; Bragança et al. 2016; Damm et al. 2012; Garibaldi et al. 2016; Hyde et al. 2009; Ivic et al. 2013; Kasson et al. 2014; Kou et al. 2014; Liu et al. 2016; Munda 2014, 2016; Oo et al. 2016; Pszczółkowska et al. 2016; Sun et al. 2012; Zhu et al. 2015). A valid *Colletotrichum* name is an urgent necessity, because *Colletotrichum* 1831, rather than *Glomerella* 1903, has been selected as the preferred holomorphic genus name (Réblová et al. 2016: 135).

This unsatisfactory situation is easily remedied under the current International Code of Nomenclature for algae, fungi, and plants (ICN (Melbourne); McNeill et al. 2012: Art. 59), in which the separation between "anamorph" and "teleomorph" names has been abolished. Here, I propose the name *Colletotrichum fioriniae*, with basionym *Glomerella acutata* var. *fioriniae*.

Taxonomy

Colletotrichum fioriniae (Marcelino & Gouli) Pennycook, **comb. & stat. nov.**

IndexFungorum IF 553097

≡ *Glomerella acutata* var. *fioriniae* Marcelino & Gouli, in Marcelino et al., Mycologia 100: 362. 2008.

Holotype: USA, New York, Westchester, Ward Pound Ridge Reserve, from mummified adult *Fiorinia externa* Ferris (*Insecta*, *Diaspididae*), "liquid-preserved perithecia (90% ethanol) produced by crossing *Colletotrichum acutatum* var. *fiorinia* [sic] strains EHS_{58} × EHS_{58} as well as mature mycelium of strain EHS_{58} in PDA agar cubes", 2005, J. Marcelino & S. Gouli (Worldwide Collection of Entomopathogenic Fungi, Entomology Research Laboratory, University of Vermont, Burlington VT, USA; ex-type culture, CBS 128517 = ARSEF 10222).

"*Colletotrichum acutatum* var. *fioriniae*" Marcelino & Gouli, Mycologia 100: 365. 2008, nom. inval. (no Latin diagnosis or description; ICN (Melbourne) Art. 39.1).

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"*Glomerella fioriniae*" R.G. Shivas & Y.P. Tan, Fungal Diversity 39: 117. 2009, nom. inval. (no full and direct reference to basionym; ICN (Melbourne) Art. 41.5).

Discussion

The validation of *Colletotrichum fioriniae* as a comb. & stat. nov. has been preferred because it recognizes the existence of the valid basionym in *Glomerella*. Alternatively, the name could have been validated as a sp. nov., since the varietal name in *Glomerella* does not have priority at specific rank.

Acknowledgments

I wish to thank Paul Kirk and Tom May for helpful discussions and for pre-submission refereeing, and Bevan Weir for advice on the International Subcommission on *Colletotrichum* Taxonomy's acceptance of *Colletotrichum* as the preferred generic name.

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Records of terricolous lichens from páramos of southern Ecuador

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Abstract—Ecological studies of five páramos in Azuay and Loja provinces recorded one lichen species new to South America (*Bryoria nitidula*), five new to Ecuador (*Cladonia halei, C. melanopoda, C. merochlorophaea, C. subreticulata, Diploschistes diacapsis*), two new to mainland Ecuador (*Cladonia grayi, C. pyxidata*), and 20 new provincial records. Brief morphological descriptions, with remarks on distribution and ecology, are provided.

Key words—Andes, biodiversity, *Cladoniaceae,* Neotropics

Introduction

The páramo is a Neotropical ecosystem located between the upper forest line and the permanent snow line. In southern Ecuador these ecosystems are found from 2800 m altitude, due to local factors such as climate, soil, and the degree of human intervention (León-Yánez 2000, Medina & Mena 2001).

The páramo flora is extremely diverse and considered the richest high mountain flora of the world (Smith & Cleef 1988, Luteyn 1999). Unfortunately, its lichen communities are relatively poorly studied (Sipman 2002a). Within the relevant published information we should emphasize the studies by Sipman (1992, 1999, 2005), which reported a total of 463 lichen species for Neotropical páramos (264 species from Colombia, 204 from Costa Rica, and 191 from

Ecuador). Also, in monographs on *Cladoniaceae* (Ahti 1992, 2000), 50 species of this family were reported from the Andean páramos and subpáramos from Costa Rica to Bolivia, 39 of them above 2800 m in páramo ecosystems in Ecuador.

Despite these previous studies, the lichen biota of Neotropical páramos still deserves further study, since the detailed distributions of these taxa are not well known (Ahti 1992, Sipman 2002a) and lichen flora in Ecuador has been poorly studied in general (Lücking 1999, Nöske & Sipman 2004, Paredes 2006). Our ongoing studies of the Ecuadorian lichen biota have compiled ca. 900 lichen species for mainland Ecuador (Cevallos 2012); comparison with the ca. 800 species reported from the Galapagos Islands (Bungartz et al. 2013) and the estimate of 2700 species for Ecuador (Lücking et al. 2009) underscores the need for further research on lichen flora in Ecuador.

Thus our goal is to contribute to the knowledge of the lichen flora in páramo ecosystems and in Ecuador overall.

Material & methods

The specimens were collected in five páramos in Loja and Azuay provinces, southern Ecuador: Cajanuma, El Cajas, Jimbura, Loma del Oro, and Punzara, located at altitudes between 2770 and 4040 m. The samples were collected during ecological studies carried out in 2010 and 2014 (González et al. 2017). Dried specimens were morphologically and anatomically examined using a stereomicroscope and compound microscope. For species identification we followed Lumbsch (1989), Ahti (2000), Brodo et al. (2001), Smith et al. (2009), and Rivas Plata et al. (2010). Secondary compounds were detected by spot tests and thin-layer chromatography (TLC). Specimen dimensions and chemotype nomenclature follow Ahti (2000). The specimens are stored in the Herbarium of Universidad Técnica Particular de Loja, Loja, Ecuador (H-UTPL).

Results

We identified one species new to South America (marked ***), five new to Ecuador (marked **), two new to mainland Ecuador (marked *), and 20 new provincial records.

Alectoria ochroleuca (Hoffm.) A. Massal., Sched. Critic. 2: 47. 1855.

Spot test: cortex KC+ yellow, PD–; medulla KC+ yellow or KC–, CK+ yellow-gold, PD–.

Specimens examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0060); 2°46′47″S 79°12′20″W, 3788 m, 13.3.2014, A. Benítez & Y.

González (H-UTPL YG-0303); 2°47′39″S 79°12′09″W, 3894 m, 26.3.2014 (H-UTPL YG-0304); 2°47′03″S 79°13′30″W, 4009 m, 15.2.2014 (H-UTPL YG-0305).

Alectoria ochroleuca is characterized by its erect to decumbent shrubby thallus with greenish yellow branches darkening towards the tips (Brodo et al. 2001).

Widespread in Asia, Central and North America, Europe, and Oceania (Guzmán-Dávalos & Álvarez 1987, Smith et al. 2009), in South America *A. ochroleuca* is distributed in Argentina, Bolivia, Chile, Colombia, Costa Rica, Ecuador, Peru, and Venezuela (Nylander 1861, Galloway & Quilhot 1998, Calvelo & Liberatore 2002, Umaña-Tenorio et al. 2002, Sipman 1999, Ramos 2014).

This species grows on the ground or rarely on shrubs and preferably in arctic-alpine ecosystems (Brodo et al. 2001). In Ecuador, it was previously cited in Chimborazo, Cotopaxi, Loja, and Pichincha provinces (Zahlbruckner 1905, 1907; Arvidsson 1991; Sklenář et al. 2010; Benítez et al. 2012). Here it is reported for the first time from Azuay province (in El Cajas páramo).

Arthrorhaphis alpina (Schaer.) R. Sant., Lichenologist 12: 106. 1980.

Specimens examined—**ECUADOR, Loja. Loja:** Punzara, 4°02′36″S 79°13′59″W, 2770 m, páramo, 19.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0105); **Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 19.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0106).

The thallus of *A. alpina* is composed of more or less rounded, convex, bullate squamules, ≤1 mm diam., yellow to yellowish green, matte, and with a more or less pruinose roughened surface. Apothecia are infrequent, located between the squamules, blackish grey-green.

Arthrorhaphis alpina is widespread in Asia, Europe, North America, and Oceania (Obermayer 1994, Esslinger & Egan 1995, Elix & McCarthy 1998, Aptroot & Feijen 2002, Aptroot & Sparrius 2003). In South America is distributed in Bolivia, Colombia, Chile, Costa Rica, Ecuador, and Venezuela (Hafellner & Obermayer 1995, Sipman 1999, Galloway & Quilhot 1998). The species grows on soil and among mosses (Smith et al. 2009).

Previously found in Ecuador in Pichincha province (Arvidsson 1991), this is the first record of *A. alpina* for Loja province.

Bryoria nitidula*** (Th. Fr.) Brodo & D. Hawksw., Op. Bot. 42: 107. 1977. Fig. 1

Fruticose, shrubby thallus, 4–8 cm, erect or spreading horizontally over the ground, branching anisotomic and usually dichotomous towards the apices. Branches dark brown to black, darker at the base, shiny and terete, 0.5–0.9 mm diam., with a few lateral perforations or fissures of 0.4–0.8 mm; lacking soredia or isidia.

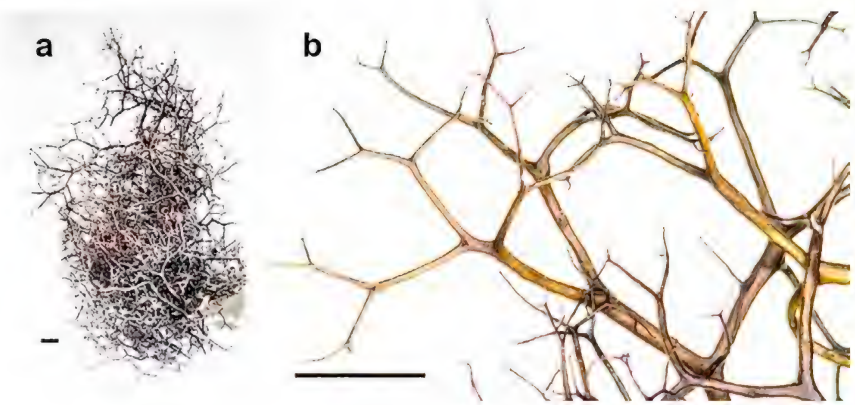

Figure 1. ***Bryoria nitidula.***
a. Thallus. b. Branching of podetia. Scale bars: a = 5 mm; b = 2 mm.

Substances detected by TLC: none.

Specimens examined—**ECUADOR**, **Azuay**. **Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0057); 2°47′39″S 79°12′09″W, 3890 m, 26.3.2014, A. Benítez & Y. González (H-UTPL YG-00301); 2°46′47″S 79°12′21″W, 3789 m, 13.3.2014 (H-UTPL YG-00298).

Bryoria nitidula occurs in Asia, Europe, and North America, growing on arctic and subarctic heaths (Smith et al. 2009). It was found for the first time in Ecuador being locally common in El Cajas páramo, at 3750–4020 m elevations. This is the first record in South America.

Our specimens closely resemble those described from North America, but the Ecuadorian samples do not contain fumarprotocetraric acid.

Cladia aggregata (Sw.) Nyl., Bull. Soc. Linn. Normandie, Ser. 2, 4: 167. 1870.

Substances detected by TLC: barbatic and 4-O-demethylbarbatic acids (chemotype I).

Specimens examined—**ECUADOR**, **Loja**. **Loja:** Parque Nacional Podocarpus, Cajanuma, 4°06′59″S 79°09′41″W, 3337 m, páramo, 29.6.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0064); 13.7.2010 (H-UTPL YG-0079); Punzara, 4°02′36″S 79°13′59″W, 2770 m, páramo, 19.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0100); **Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 19.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0005 and H-UTPL YG-0006); **Espíndola:** Jimbura, 4°42′37″S 79°25′46″W, 3450 m, páramo, 8.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0082 and H-UTPL YG-0083).

The thallus of *C. aggregata* is characterized by its dark brown to blackish, pale yellowish to straw-colored or pale green pseudopodetia, 5–150 × 0.5–8 mm.

The pseudopodetial wall is cartilaginous with infrequent to abundant rounded to elliptic perforations; the central canal surface is white and loosely arachnoid (Ahti 2000).

Although several species have been described with the same chemosyndrome within the *Cladia aggregata* complex (Parnmen et al. 2012, 2013), their distribution and morphology do not match our samples. Moreover, the barcoding molecular sequences produced for our samples (nuITS region) place our samples with *Cladia aggregata* s. str.

Cladia aggregata s. lat. is distributed in the Southern Hemisphere, including Australasia, Southeast Asia to South Japan and India, and South Africa (Ahti 2000). In the Neotropical region it is widespread, from Mexico and the West Indies to the subantarctic islands, but it is absent in lowland and arid regions (Ahti 2000). It is very common in montane areas, on soil, and wood (Ahti 2000). In Ecuador four chemotypes (II, III, IV, and VII) have previously been reported (Ahti 2000). Chemotype I was found for the first time in mainland Ecuador growing in páramos of Azuay and Loja provinces. This chemotype was previously reported in Galapagos Islands (Yánez-Ayabaca et al. 2013). Our specimens constitute the first records from Loja province.

Cladia fuliginosa Filson, Victorian Nat. 87: 325. 1970

Substances detected by TLC: divaricatic and usnic acids.

Specimens examined—**ECUADOR, Loja. Loja:** Parque Nacional Podocarpus, Cajanuma, 4°06′59″S 79°09′41″W, 3337 m, páramo, 29.6.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0081); Punzara, 4°02′36″S 79°13′59″W, 2770 m, páramo, 19.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0098 and H-UTPL YG-0099); **Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 7.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0001 and H-UTPL YG-0002); **Espíndola:** Jimbura, 4°42′37″S 79°25′46″W, 3450 m, páramo, 8.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0091).

Cladia fuliginosa produces green to blackish brown pseudopodetia, ≤70 × 2 mm; the perforated and irregularly branched pseudopodetia are mostly filled by a dark brown to black inner medulla (Ahti 2000).

Distributed throughout the Andes, from Colombia to Peru growing on soil in páramos, *C. fuliginosa* is also present in Australia and New Zealand (Ahti 2000). In Ecuador, it has previously been cited in Azuay, Imbabura, and Pichincha provinces (Arvidsson 1991, Ahti 2000). Our collections constitute the first records of *C. fuliginosa* from Loja province.

Cladonia aleuropoda Vain., Beibl. Hedwigia 38: 190. 1899.

Substances detected by TLC: fumarprotocetraric and often quaesitic acids (chemotype I) and grayanic acid (chemotype II).

Specimen examined—**ECUADOR, Loja. Loja:** Punzara, 4°02′36″S 79°13′59″W, 2770 m, páramo, 19.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPLYG-0104).

The thallus produces 3–10 cm tall unbranched or sparsely branched whitish to brownish gray podetia. Initially subulate, the podetial tips later produce 1–7 mm wide scyphi (Ahti 2000).

Cladonia aleuropoda is distributed at 2200–4500 m elevations along the Andes in Colombia, Costa Rica, Ecuador, Guatemala, México, Peru, and Venezuela, growing on soil and rocks in páramos and upper cloud forests (Ahti 2000). Chemotypes I and II have been previously recorded in Ecuador from Azuay, Carchi, Chimborazo, Cotopaxi, Napo, and Pichincha provinces (Ahti 2000). This is the first record of chemotype I for Loja province.

Cladonia arbuscula (Wallr.) Flot., Thermen Warmbr. Riesengeb.: 94. 1839, s.lat.

Substances detected by TLC: fumarprotocetraric and usnic acids (chemotype I).

Specimens examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0041); 2°46′60″S 79°13′61″W, 4012 m, 22.2.2014 (H-UTPL YG-0275); 2°47′40″S 79°12′08″W, 3893 m (H-UTPL YG-0278); 2°47′39″S 79°12′09″W, 3891 m, 26.3.2014 (H-UTPL YG-0274); 2°46′47″S 79°12′19″W, 3789 m, 14.3.2014 (H-UTPL YG-0276); 2°46′50″S 79°12′20″W, 3787 m, 13.3.2014 (H-UTPL YG-0273). **Loja. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 19.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0037); **Espíndola:** Jimbura, 4°42′37″S 79°25′46″W, 3450 m, páramo, 8.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0092).

Under *C. arbuscula,* we include specimens representing the type subspecies and the subspecies *boliviana*. Piercey-Normore et al. (2010), who demonstrated that molecular data do not support the existence of some subspecies, did not include subsp. *boliviana*, and so additional molecular analyses are needed to test the status of subsp. *boliviana*.

C. arbuscula subsp. *boliviana* (Ahti) Ahti & DePriest is characterized by yellowish to whitish gray podetia with slightly brownish tips. It grows up to 17 cm, its podetia are slender, generally loosely branched, with its ultimate branchlets erect to divaricate, rather dense at apex but rarely strongly unilaterally deflexed (Ahti 2000). The subspecies differs from *C.* subsp. *arbuscula* mainly in its darker color, bigger size, and highly frequent dichotomous branching, and the absence of psoromic acid (Ahti 2000). However, these characters could be misleading and molecular data are necessary for the identification of the Andean material (Ahti pers. comm.).

Cladonia arbuscula is common in páramos and upper Andean forests (Ahti 2000) in Bolivia (chemotype I), Brazil (I, II), Colombia (I, II, III), and Venezuela (I, II, III) but rare in Costa Rica (II) and Guatemala (I). In Ecuador chemotype

II (with usnic acid only) has been previously found in the provinces of Azuay, Galapagos Islands, Imbabura, Loja, and Tungurahua (Ahti 2000). Chemotype I was found for the first time in Ecuador, growing in three different páramos.

Cladonia coccifera (L.) Willd., Fl. Berol. Prodr.: 361. 1787.

Substances detected by TLC: zeorin and usnic acid.

Specimens examined—**ECUADOR**, **Azuay**. **Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0052 and H-UTPL YG-0053); 2°46′46″S 79°12′20″W, 3786 m, 13.3.2014, A. Benítez & Y. González (H-UTPL YG-0160).

Cladonia coccifera is characterized by its persistent primary thallus, consisting of ascending squamules, often with orange basal parts. The 0.7–3.5 cm tall podetia are greenish, yellowish or whitish gray, with an areolate-corticate surface and totally covered by pruinose granules, schizidia, and phyllidia (also inside the scyphi). Red-coloured hymenial discs are fairly common (Ahti 2000).

Distributed in the Northern Hemisphere in Himalaya, Java, and Papua New Guinea, *C. coccifera* has been found at elevations of 2400–4300 m throughout Central and South America in Argentina, Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Guatemala, Hispaniola, Venezuela, and Peru (Sipman 1999, Ahti 2000, Calvelo & Liberatore 2002, Flakus et al. 2008), growing on humous soils and occasionally on wood and plant debris. Previously reported for Ecuador in Carchi, Cotopaxi, Loja, and Zamora-Chinchipe (Ahti 2000, Nöske & Sipman 2004, Mandl 2007), this is the first record of *C. coccifera* for Azuay province.

Cladonia corniculata Ahti & Kashiw., Stud. Cryptog. South. Chile: 136. 1984.

Substances detected by TLC: fumarprotocetraric acid.

Specimens examined—**ECUADOR**, **Loja**. **Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 19.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0028); **Loja:** Parque Nacional Podocarpus, Cajanuma, 4°06′59″S 79°09′41″W, 3337 m, páramo, 13.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0067, H-UTPL YG-0068, H-UTPL YG-0069 and H-UTPL YG-0080).

Cladonia corniculata is characterized by 12–60 × 0.5–2.5 mm white, green, or whitish gray podetia. Podetia are flexuous or not, at first unbranched and later dichotomously branched, with subulate tips, always ascyphose, sometimes with obtuse or dilated branchlets. Thallus surface is completely ecorticate with farinose to slightly granulose soredia (Ahti 2000).

Distributed throughout Asia, Africa, Australia, New Zealand, and the subantarctic islands, *C. corniculata* is widespread in Central and South America (especially at high elevations in the Andes). It grows on moist and shady road banks in páramos being rare on bare soils (Stenroos et al. 1992, Ahti 2000).

In Ecuador the species was previously reported from Azuay, Carchi, Cotopaxi, Galapagos Islands, Imbabura, Napo, and Pichincha (Ahti 2000); our collections represent the first records for Loja province.

Cladonia crispata (Ach.) Flot., Thermen Warmbr. Riesengeb.: 93. 1839.

Substances detected by TLC: squamatic acid.

Specimen examined—**ECUADOR, Loja.** Loja: Punzara, 4°02′36″S 79°13′59″W, 2770 m, páramo, 19.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0097).

The thallus of *C. crispata* is characterized by its 20–80 × 0.5–2 mm brownish gray podetia that are not blackish at the base. The podetia have dilated apices, 1–3 mm wide axillary funnels, and a smooth surface (Ahti 2000).

A subcosmopolitan species, distributed throughout Australasia, East Africa, Asia, Europe, New Guinea, and North America (Ahti 2000, Burgaz & Ahti 2009), *C. crispata* has been recorded throughout Central and South America in Colombia, Costa Rica, Dominican Republic, Ecuador, Jamaica, Peru, and Venezuela (Ahti 2000) as locally common in páramos and upper Andean forests where it grows on soil and wood at 1700–3300 m altitudes (Ahti 2000). Previously recorded in Ecuador from Zamora-Chinchipe (Nöske & Sipman 2004) in upper montane forests, our first collection in páramo in Loja province widens the distribution and ecology of *Cladonia crispata* in Ecuador.

Cladonia grayi* G. Merr. ex Sandst., Sandstede Clad. Exs.: no. 1847. 1929. Fig. 2

Podetia 1–2 cm, scyphose, greenish gray, often brownish in basal parts, with proliferations from scyphal margins, scyphi 2–4.5 mm diam. Surface rough, verruculose, upper part and interior of scyphi granulose, clearly sorediate or eroded in some parts, with age developing macrosquamules. Hymenial discs frequent, dark brown.

Substances detected by TLC: fumarprotocetraric and grayanic acids.

Specimen examined—**ECUADOR, Azuay.** Cuenca: Parque Nacional El Cajas, 2°46′47″S 79°14′57″W, 3789 m, páramo, 13.3.2014, A. Benítez & Y. González (H-UTPL YG-0165).

Cladonia grayi, widespread in the Northern Hemisphere from the Arctic to temperate regions (where it is very common in eastern North America) and is also present in Australasia (Ahti 2000, Burgaz & Ahti 2009). In the Neotropical region it is scattered throughout Argentina, Bolivia, Brazil, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Galapagos Islands, Guatemala, Haiti, Honduras, Jamaica, Paraguay, Uruguay, and Venezuela, growing at high elevations on highly acidic soils (Ahti 2000, Burgaz & Ahti 2009, Yánez-Ayabaca et al. 2013). Our collection from one locality in Azuay province represents a first record of *C. grayi* from mainland Ecuador.

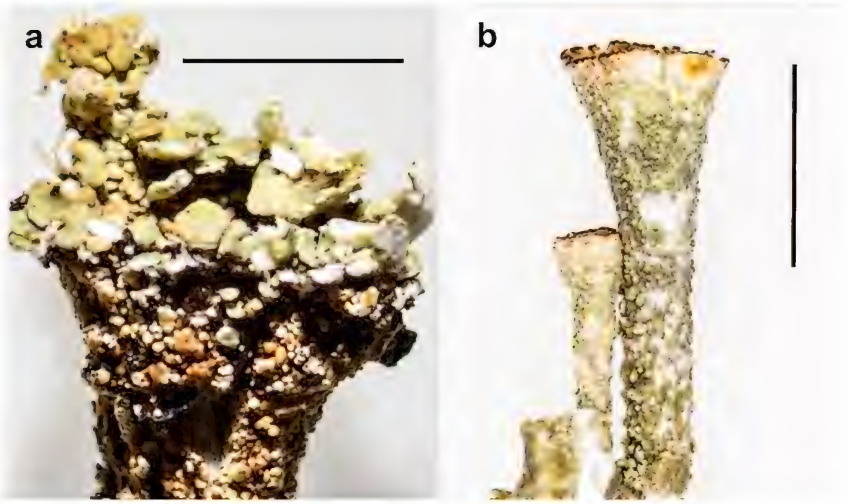

FIGURE 2. ***Cladonia grayi.*** a. Macrosquamules on margin of scyphi. b. Granules on upper part of scyphi. Scale bars: a = 3 mm; b = 2 mm.

Although similar to specimens described for the Neotropical and temperate regions (Ahti 2000), our Ecuadorian material is does not develop macrosquamules at the base and is found at higher altitudes (3789 m).

Cladonia halei** (Ahti) Ahti & DePriest, Mycotaxon 78: 501. 2001. FIG. 3

Podetia ash gray or brownish gray, darkening when exposed. Thallus ≤12 cm tall, with anisotomic branches, usually deflexed, with clearly distinct 0.6–2 mm thick main axis. Podetia surface markedly verruculose with arachnoid interspaces.

Substances detected by TLC: fumarprotocetraric acid.

SPECIMENS EXAMINED—**ECUADOR**, **AZUAY**. **Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0050 and H-UTPL YG-0051); 2°47′08″S 79°13′32″W, 4032 m, 8.2.2014, A. Benítez & Y. González (H-UTPL YG-0299); 2°47′38″S 79°12′09″W, 3884 m, 25.3.2014 (H-UTPL YG-0286); 2°46′46″S 79°12′20″W, 3788 m, 13.3.2014 (H-UTPL YG-0300).

Cladonia halei has been previously recorded in Colombia, Papua New Guinea, Peru, and Venezuela (Ahti 2000 and pers. comm.), growing in páramo and subpáramo zones at 2500–4000 m altitudes in wet *Sphagnum* bogs and on drier ground in humid montane regions (Ahti 2000). Our discovery of *C. halei* for the first time in Ecuador growing in El Cajas páramo considerably widens its world distribution.

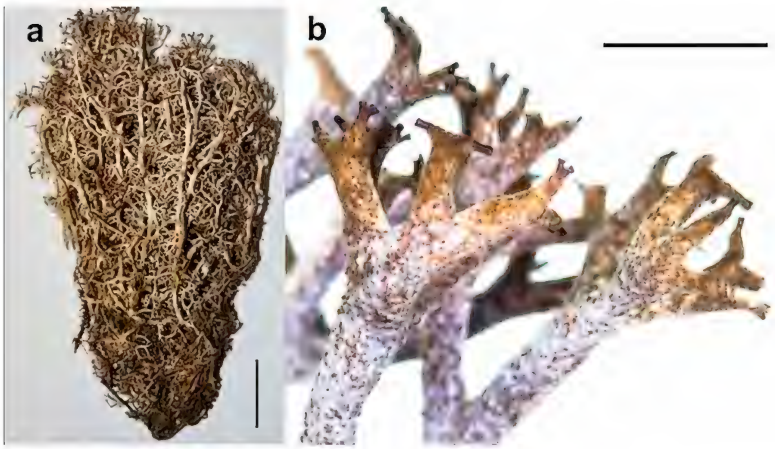

Figure 3. *Cladonia halei.*
a. Thallus. b. Branching of podetia. Scale bars = 5 mm.

Our specimens are similar in morphology, chemistry, and ecology to that described for the Neotropical region.

Cladonia leprocephala Ahti & S. Stenroos, Ann. Bot. Fenn. 23: 236. 1986.

Substances detected by TLC: thamnolic acid.

Specimens examined—**ECUADOR**, **Azuay**. **Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0046); 2°47′37″S 79°12′09″W, 3891 m, 25.3.2014, A. Benítez & Y. González (H-UTPL YG-0166).

Cladonia leprocephala is characterized by its 8–23 × 0.8–2 mm greenish to whitish gray podetia with scyphi having subentire margins 2–3 mm wide. Thallus surface in generally basally areolate-corticate up to the scyphi, with granulose soredia. The red hymenial discs are rare and present at the tips of proliferations from scyphal margins (Ahti 2000).

Distributed along the Andes in Colombia, Costa Rica, Ecuador, Peru, and Venezuela in páramos at 2900–4250 m elevations, growing on moist and humous soils, on wood, and as epiphyte on shrubs at timberline (Ahti 2000, pers. comm.), in Ecuador *C. leprocephala* has been previously reported from Cotopaxi, Napo, Pichincha, and Tungurahua (Ahti 2000). Our collections represent the first records of the species for Azuay province.

Cladonia lopezii S. Stenroos, Ann. Bot. Fenn. 26: 250. 1989.

Substances detected by TLC: didymic acid.

Specimen examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0106).

The species has ≤25 × 30 mm brown podetia that are marginal on primary squamules, which elongate and roll up to form the podetial tube, abruptly flaring to form conspicuously dorsiventrally flattened web-like structures. The podetia usually produce red hymenial discs, forming agglomerations up to 9 mm wide (Ahti 2000).

Cladonia lopezii occurs in the high Andes from Bolivia to Venezuela and in Brazil at 1200–3935 m altitudes, growing on soil, rocks, and rotten wood (Ahti 2000). The species has been previously found in Loja, Morona-Santiago, and Zamora-Chinchipe (Ahti 2000, Nöske & Sipman 2004, Mandl 2007); our collection constitutes the first record for Azuay province.

Cladonia macilenta Hoffm., Deutschl. Fl. 2: 126. 1796.

Substances detected by TLC: barbatic acid [chemotype II].

Specimen examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′46″S 79°12′18″W, 3793 m, páramo, 14.3.2014, A. Benítez & Y. González (H-UTPL YG-0199).

The thallus of *C. macilenta* is characterized its 5–40 × 0.3–2.5 mm whitish ascyphose podetia with subulate to blunt apices that are swollen below the hymenia, unbranched or sometimes branched (Ahti 2000, Burgaz & Ahti 2009). The thallus surface is mostly covered by abundant farinose-soredia (Ahti 2000, Burgaz & Ahti 2009).

Substances detected by TLC: barbatic acid. There are two major chemotypes recognized, chemotype I with thamnolic acid as major compound, and chemotype II with barbatic acid as major compound.

Rare in tropical lowlands and highest mountains, *C. macilenta* is widespread between 1500–3250 m along the northern Andes, growing on wood, stumps, tree bases, peat bogs, acid rocks, and humous or sandy acid soils (Ahti 2000, Burgaz & Ahti 2009). In Ecuador it has been previously found in Carchi (chemotype II), Galapagos Islands (chemotypes I and II), and Tungurahua (chemotype II) (Ahti 2000, Yánez-Ayabaca et al. 2013). Our specimen (chemotype II) represents a first record for Azuay province.

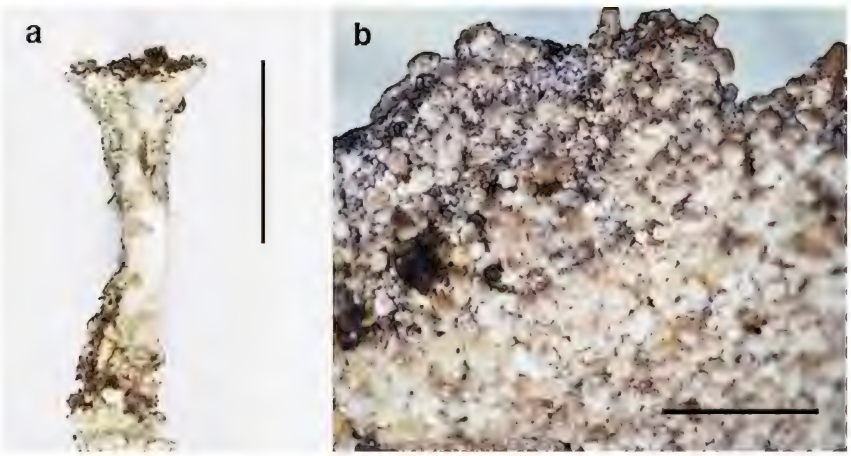

FIGURE 4. a. ***Cladonia melanopoda.*** Small squamules on surface of podetium. Scale bar = 2 mm. b. ***Cladonia merochlorophaea.*** Phyllidia and schizidia inside scyphi. Scale bar = 2 mm.

Cladonia melanopoda ** Ahti, Symb. Bot. Upsal. 32(1): 7. 1997. FIG. 4a

Podetia 45–60 × 0.5–1 mm, slender, unbranched to slightly branched, brownish to blackish at the base. The podetial surface either totally decorticated or with areolate schizidia or small squamules. Scyphi 1–4 mm wide, always with central proliferations in 5–7 tiers.

Substances detected by TLC: fumarprotocetraric acid and traces of quaesitic acid.

SPECIMENS EXAMINED—**ECUADOR, LOJA. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 19.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0019, H-UTPL YG-0020, H-UTPL YG-0021).

Previously found at high elevations of the Andean range in Argentina, Bolivia, and Peru on soil in páramo and timberline vegetation at 1300–4000 m (Ahti 2000), *C. melanopoda* is reported here for the first time in Ecuador from one páramo in Loja province.

Our specimens are very close to those described from the Neotropical region.

Cladonia merochlorophaea ** Asahina, J. Jap. Bot. 16: 713. 1940. FIG. 4b

Podetia 10–30 × 1–2 mm, scyphose with 2–6 mm wide scyphi, sometimes simple or frequently with proliferations from margins and the old bases strongly blackish. The surface is usually verruculose with wide bare spaces

and macrosquamules. Inside scyphi there are phyllidia and schizidia often brownish. The scyphal margin may produce pyriform pycnidia.

Substances detected by TLC: merochlorophaeic and fumarprotocetraric acids.

Specimens examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′60″S 79°13′31″W, 4007 m, páramo, 22.2.2014, A. Benítez & Y. González (H-UTPL YG-0151); 2°47′38″S 79°12′10″W, 3895 m, 25.3.2014 (H-UTPL YG-0155); 2°47′36″S 79°12′10″W, 3895 m (H-UTPL YG-0158); 2°46′47″S 79°12′21″W, 3789 m, 13.3.2014 (H-UTPL YG-0162); 2°46′46″S 79°12′19″W, 3790 m, 14.3.2014 (H-UTPL YG-0290).

A subcosmopolitan species found from arctic to temperate regions in Antarctic, Australasia, Eurasia, North America, and southern South America, *C. merochlorophaea* is found In the Neotropical region in Bolivia, Brazil, Peru, and Venezuela (Ahti 2000, pers. comm.) usually growing on acid humus, peat, and wood (Stenroos et al. 1992, Ahti 2000, Burgaz & Ahti 2009). Our collections constitute the first reports for the species in Ecuador, where it was being locally common in Azuay province (in El Cajas páramo).

Our specimens are similar in morphology, chemistry, and ecology to that described for the Neotropical and temperate regions (Ahti 2000).

Cladonia pyxidata* (L.) Hoffm., Deutschl. Fl. 2: 121. 1796. Fig. 5

Primary thallus persistent, squamules 1–3 × 2–3 mm. Scyphose podetia 4–12 mm tall, greenish gray to dark brown, stalks 1–1.5 mm diam., scyphi 0.8–4.5 mm diam., usually simple or sometimes proliferating from margins. Podetia surface corticated, often continuous but can be areolate or cracked and usually decorticated above. Inside of scyphi it presents schizidia, phyllidia, microsquamules, and flat granules, these latter usually larger.

Substances detected by TLC: fumarprotocetraric and homosekikaic acids (chemotype II).

Specimens examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°47′37″S 79°12′09″W, 3889 m, páramo, 25.3.2014, A. Benítez & Y. González (H-UTPL YG-0168); 2°46′47″S 79°12′21″W, 3791 m, 13.3.2014 (H-UTPL YG-0164 and H-UTPL YG-0167).

Distributed throughout Africa, Asia, Australia, Europe, North America, and New Zealand (Smith et al. 2009), in the Neotropical region *C. pyxidata* has been previously found in Argentina, Brazil, Colombia, Dominican Republic, El Salvador, Galapagos Islands, Haiti, Honduras, Guatemala, Jamaica, Peru, Uruguay, and Venezuela, growing on acid substrates in bare and humous soils, mossy rocks, and rarely on dead wood (Ahti 2000, pers. comm., Burgaz & Ahti 2009, Yánez-Ayabaca et al. 2013). Our collection from Azuay

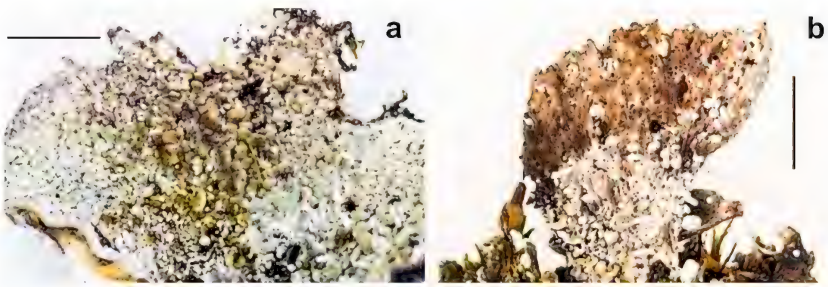

FIGURE 5. ***Cladonia pyxidata.*** a. Phyllidia, schizidia, microsquamules, and flat granules inside scyphi. b. Decorticate podetia surface. Scale bars = 3 mm.

province (in El Cajas páramo) represents the first record of *C. pyxidata* for mainland Ecuador. Chemotype II is fairly rare and has previously been noted only for Guatemala and Honduras (Ahti 2000).

The Ecuadorian material is similar in morphology and ecology to those described for the Neotropical region (Ahti 2000, Burgaz & Ahti 2009, Yánez-Ayabaca et al. 2013) but in Ecuadorian samples the scyphi are wider (≤4 mm).

Cladonia squamosa (Scop.) Hoffm., Deutschl. Fl. 2: 125. 1796.

Substances detected by TLC: squamatic acid (chemotype I), thamnolic and barbatic acids (chemotype II).

SPECIMENS EXAMINED—**ECUADOR, LOJA. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 7.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0017); **Loja:** Parque Nacional Podocarpus, Cajanuma, 4°06′59″S 79°09′41″W, 3337 m, páramo, 13.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0066); **AZUAY. Cuenca:** Parque Nacional El Cajas, 2°47′38″S 79°12′09″W, 3885 m, páramo, 25.3.2014, A. Benítez & Y. González (H-UTPL YG-0193); 2°47′37″S 79°12′09″W, 3891 m, 26.3.2014 (H-UTPL YG-0194); 2°47′39″S 79°12′09″W, 3894 m, 26.3.2014 (H-UTPL YG-0195); 2°47′38″S 79°12′09″W, 3886 m, 25.3.2014 (H-UTPL YG-0197); 2°46′47″S 79°12′19″W, 3782 m, 25.3.2014 (H-UTPL YG-0198); 2°46′37″S 79°12′09″W, 3890 m, 25.3.2014 (H-UTPL YG-0202); 2°47′37″S 79°12′09″W, 3889 m, 25.3.2014 (H-UTPL YG-0203); 2°47′37″S 79°12′09″W, 3889 m, 25.3.2014 (H-UTPL YG-0204); 2°46′46″S 79°12′20″W, 3733 m, 13.3.2014 (H-UTPL YG-0207); 2°47′08″S 79°13′32″W, 4030 m, 9.2.2014 (H-UTPL YG-0209); 2°47′37″S 79°12′04″W, 3889 m, 25.3.2014, (H-UTPL YG-0210); 2°47′00″S 79°13′31″W, 4018 m, 22.2.2014 (H-UTPL YG-0211); 2°47′38″S 79°12′09″W, 3880 m, 25.3.2014 (H-UTPL YG-0212); 2°47′37″S 79°12′10″W, 3886 m, 25.3.2014 (H-UTPL YG-0213); 2°46′47″S 79°12′19″W, 3782 m, 14.3.2014 (H-UTPL

YG-0214); 2°47′08″S 79°13′32″W, 4029 m, 9.2.2014 (H-UTPL YG-0215); 2°47′03″S 79°13′32″W, 4006 m, 16.2.2014 (H-UTPL YG-0221); 2°47′38″S 79°12′10″W, 3891 m, 25.3.2014 (H-UTPL YG-0222); 2°47′38″S 79°12′09″W, 3883 m, 25.3.2014 (H-UTPL YG-0236); 2°46′47″S 79°12′21″W, 3788 m, 13.3.2014 (H-UTPL YG-0258).

In *Cladonia squamosa,* the podetia are 30–140 × 0.5–5 mm, greenish gray to strongly brown, not black at the base, unbranched or irregularly branched, with usually dilated axils and perforated apex (Ahti 2000, Burgaz & Ahti 2009). The thallus surface is never sorediate, but mostly or totally decorticated and densely covered with crenulate or laciniate 1–8 mm tall squamules and granules (Ahti 2000).

Widespread in temperate and cooler regions in both Hemispheres, also along the Andes, from Mexico to Chile, and Argentina at 1000–4500 m altitudes, *C. squamosa* is also present in southeast Brazil down to 700 m. It grows in cloud forests and moist páramos, on rocks and acidic and humous soils or wood (Ahti 2000, Burgaz & Ahti 2009). In Ecuador, the species has been previously found in Azuay (chemotype I), Carchi (chemotype I), Pastaza (chemotype II), and Zamora-Chinchipe (chemotype II) provinces (Arvidsson 1991, Ahti 2000, Nöske & Sipman 2004). Our collections represent the first records of chemotype II specimens from Azuay and Loja provinces and the first record of chemotype I for Azuay province.

Cladonia subreticulata** Ahti, Acta Bot. Fenn. 10: 168. 1973. FIG. 6a

Podetial surface compact and slightly arachnoid, usually forming well-developed cushions. Podetia 30–50 × 3.5–4.5 mm, thick, yellow to grayish yellow, with very irregular branching, open apices and axils, and perforated podetial wall.

Substances detected by TLC: usnic acid (chemotype III).

SPECIMENS EXAMINED—**ECUADOR, LOJA. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″ W, 3245 m, páramo, 19.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0023 and H-UTPL YG-0024).

Previously reported from South America in Brazil, Guyana, Peru, and Venezuela at high elevations in woodlands and cloud forests on sandy soils and rock outcrops (Ahti 2000, Ahti & Sipman 2013), *C. subreticulata* was found for the first time in Ecuador, where it was very rare found growing on relatively bare soils in Loma del Oro páramo in Loja province.

Our specimens, which morphologically, ecologically, and chemically resemble those described for the Neotropic Region (Ahti 2000), differ in having smaller (≤5 cm) podetia.

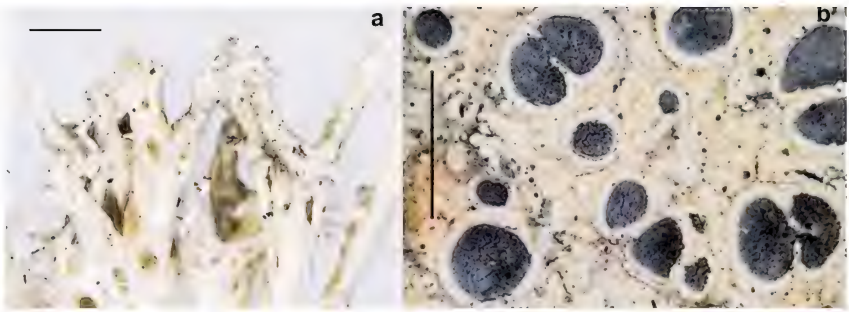

FIGURE 6. a. ***Cladonia subreticulata.*** Branching of podetia. Scale bar = 3 mm.
b. ***Diploschistes diacapsis.*** Apothecia. Scale bar: 3 mm.

Dibaeis columbiana (Vain.) Kalb & Gierl, Herzogia 9: 621. 1993.

SPECIMEN EXAMINED—**ECUADOR, LOJA. Espíndola:** Jimbura, 4°42′37″S 79°25′46″W, 3450 m, páramo, 8.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0088).

Diabaeis columbiana is characterized by a densely granular crustose thallus with 0.2 mm wide pale gray granules, 2–11 mm tall podetia with convex pink apothecia, and lacking schizidia or soredia (Sipman 1997).

A terricolous species found in Bolivia, Colombia, Costa Rica, Ecuador, and Venezuela (Marcano et al. 1996, Sipman 1999, Flakus & Wilk 2006), in Ecuador *D. columbiana* was previously found on subalpine dwarf-forest and páramo in Zamora-Chinchipe (Nöske & Sipman 2004). Our collection is the first record for Loja province, where it is rare.

Diploschistes diacapsis** (Ach.) Lumbsch, Lichenologist 20: 20. 1988. FIG. 6b

Thallus verrucose and areolate, upper surface whitish to whitish-gray, slightly to abundantly pruinose; apothecia urceolate, 0.5–2.5(–4) mm diam. black or pruinose gray; ascospores 4–8 per ascus.

SPECIMENS EXAMINED—**ECUADOR, LOJA. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 19.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0034); **AZUAY. Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010 (H-UTPL YG-0058); 2°46′46″S 79°12′21″W, 3790 m, 13.3.2014, A. Benítez & Y. González (H-UTPL YG-0302).

Diploschistes diacapsis is identified by the K+ yellow reaction of the thallus due to the presence of diploschistesic acid as major constituent (Rivas Plata et al. 2010); the PD–, C+ red and KC+ red chemistry is due to lecanoric and diploschistesic acids.

Found in Africa, Asia, Australia, Europe, North, and South America (Lumbsch 1989), *D. diacapsis* has been reported from South America from páramo ecosystems in Chile, Costa Rica, and Peru (Lumbsch 1989, Galloway & Quilhot 1998, Sipman 1999). Our collections constitute the first records from Ecuador, where it was found growing in two different páramos in Azuay and Loja provinces.

Our specimens closely resemble those described in the Holarctic zone (Lumbsch 1989) except for its larger (≤4 mm) apothecia.

Normandina pulchella (Borrer) Nyl., Ann. Sci. Nat., Bot., sér. 4, 15: 382. 1861.

Specimens examined—**ECUADOR, Loja. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 13.8.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0107); **Espíndola:** Jimbura, 4°42′37″S 79°25′46″W, 3450 m, páramo, 8.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0108).

Normandina pulchella is characterized by its very thin 0.7–2.5 mm tall scattered to crowded green to slightly bluish green squamules with sharply raised margins. Soralia are often present on lobe surface and margins. The lower squamule surfaces are whitish and slightly felted with numerous hyphae (Smith et al. 2009).

A frequent cosmopolitan species (Smith et al. 2009), in the Neotropical region *N. pulchella* is found in Argentina, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, Guyana, Peru, Uruguay, and Venezuela (Calvelo & Liberatore 2002, Flakus & Wilk 2006, Sipman 1999). It grows on mosses, other lichens, bark, and rocks in habitats with high air humidity (Nash et al. 2004). In Ecuador the species was previously found in Galapagos Islands and Zamora-Chinchipe (Arvidsson 1991, Elix et al. 1998, Davey 1999, Nöske 2004, Nöske & Sipman 2004, Mandl 2007). Our collections represent the first records from Loja province, where it was found growing on bryophytes.

Phyllobaeis imbricata (Hook.) Kalb & Gierl, Herzogia 9: 610. 1993.

Specimens examined—**ECUADOR, Loja. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 7.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0029, H-UTPL YG-0030 and H-UTPL YG-0031); **Loja:** Parque Nacional Podocarpus, Cajanuma, 4°06′59″S 79°09′41″W, 3337 m, páramo, 29.6.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0073 and H-UTPL YG-0074).

Phyllobaeis imbricata is distinguished by its squamulose primary thallus with long deeply lobed squamules, cylindric podetia, and pink terminal apothecia.

A high-elevation Neotropical species in Bolivia, Brazil, Colombia, Costa Rica, Ecuador, and Venezuela (Gierl et al. 1993, Sipman 1999, Umaña-Tenorio et al. 2002, Flakus & Wilk 2006), in Ecuador, *P. imbricata* was previously found

in Zamora-Chinchipe on subalpine dwarf-forest and páramo (Nöske & Sipman 2004). Our specimens represent the first records for Loja province where they were collected from two different páramos.

Stereocaulon obesum Th. Fr., Stereoc. Piloph. Comm.: 28. 1857.

Spot test: K+ yellow, PD– or slowly yellow. Substances detected by TLC: atranorin and lobaric acid.

Specimen examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0056).

Stereocaulon obesum is characterized its abundant and small upright and thin pseudopodetia, unbranched at the base, but branched toward the apex, bicolored peltate phyllocladia, and inconspicuous or irregular translucent cephalodia (Rincón-Espitia & Mateus 2013).

Restricted to the Neotropics at 1700–4000 m altitudes in Costa Rica, Ecuador, Guatemala, and Panamá (Sipman 2002b), in Ecuador *S. obesum* was previously found in Pichincha (Arvidson 1991). Our collection constitutes the first record for Azuay province.

Stereocaulon ramulosum Raeusch., Nomencl. Bot., Ed. 3: 328. 1797.

Spot test: K+ yellow, PD+ slowly yellow. Substances detected by TLC: atranorin and perlatolic acid.

Specimen examined—**ECUADOR, Loja. Saraguro:** Loma del Oro, 3°40′52″S 79°14′24″W, 3245 m, páramo, 7.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0039).

Stereocaulon ramulosum is characterized by its >20 mm tall pseudopodetia unbranched at the base with a few branches towards the apex and with few more or less branched cylindrical phyllocladia, abundant large convex reddish brown to black apothecia, and globose wrinkled cephalodia (Sipman 2002b, Rincón-Espitia & Mateus 2013).

Widespread in the Southern Hemisphere and in Neotropical mountains at 1000–4500 m altitudes in Brazil, Bolivia, Colombia, Costa Rica, Cuba, Dominican Republic, Ecuador, Mexico, Peru, and Venezuela (Sipman 2002b), in Ecuador *S. ramulosum* was previously recorded from Chimborazo, Loja, Pastaza, Pichincha, and Zamora-Chinchipe provinces (Mitten 1851, Leighton 1866, Müller 1879, Zahlbruckner 1905, 1907, Nöske & Sipman 2004). Our collection represents the first record for Loja province.

Stereocaulon tomentosum Fr., Sched. Crit. Lich. Suec. Exs. 3: 20. 1825.

Spot test: PD+ orange, K+ orange. Substance detected by TLC: atranorin and stictic acid, corresponding to the type variety.

Specimens examined—**ECUADOR, Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0061); 2°47′03″S 79°13′30″W, 4009 m, páramo, 15.2.2014, A. Benítez & Y. González (H-UTPL YG-0308).

Stereocaulon tomentosum is characterized by an erect to prostrate thallus, with lobed, squamulose, warty, or flattened phyllocladia (Brodo et al. 2001), pseudopodetia covered with a thick felt layer (white tomentum) that also covers the cephalodia, and numerous small (<1 mm diam) apothecia sitting on short subapical branchlets and with spherical cephalodia (Sipman 2002b).

A subcosmopolitan species widespread in the Neotropics at 2300–4400 m altitudes in Bolivia, Colombia, Costa Rica, Dominican Republic, Ecuador, Mexico, Peru, and Venezuela (Sipman 2002b), in Ecuador *S. tomentosum* was previously found in Chimborazo, Pichincha, and Zamora-Chinchipe (Leighton 1866, Arvidsson 1991, Nöske & Sipman 2004). Our collections are the first records for Azuay province.

Thamnolia vermicularis (Sw.) Schaer., Enum. Crit. Lich. Eur.: 243. 1850.

Spot test: PD+ yellow-orange, K+yellow-orange, UV–, with thamnolic acid.

Specimens examined—**ECUADOR, Loja. Loja:** Parque Nacional Podocarpus, Cajanuma, 4°06′59″S 79°09′41″W, 3337 m, páramo, 29.6.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0077); **Espíndola:** Jimbura, 4°42′37″S 79°25′46″W, 3450 m, páramo, 8.7.2010, A. Benítez, Y. González & M. Prieto (H-UTPL YG-0109); **Azuay. Cuenca:** Parque Nacional El Cajas, 2°46′53″S 79°13′21″W, 3930 m, páramo, 27.7.2010, A. Benítez, Y. González & M. Prieto; 2°47′03″S 79°13′30″W, 4006 m, páramo, 15.2.2014, A. Benítez & Y. González (H-UTPL YG-0306); 2°47′36″S 79°12′09″W, 3890 m, 26.3.2014 (H-UTPL YG-0307).

Thamnolia vermicularis is characterizes as a fruticose species with a 20–70 × 1–2.5 mm decumbent to erect thallus with unbranched to slightly branched hollow white to cream white stalks that are pointed at the tips, and which lacks soredia, isidia, and fruiting bodies (Brodo et al. 2001).

The molecular study of *Thamnolia* Platt & Spatafora (2000) detected enough genetic differences separating *T. vermicularis* (containing thamnolic acid) and *T. subuliformis* (containing squamatic and baeomycesic acids and with a UV+Y cortex and a UV+ blue-white medulla) to support them as independent species.

Widespread in Australia, Asia, Europe, New Zealand, and North America (Smith et al. 2009), *T. vermicularis* has been recorded at 2800–4550 m altitudes in the Neotropical region from Argentina, Bolivia, Chile, Colombia, Costa Rica, Ecuador, Peru, and Venezuela (Soukup 1965, Sipman 1999, Calvelo & Liberatore 2002, Flakus & Wilk 2006, Villagra et al. 2009). In Ecuador, it was previously found in Chimborazo, Cotopaxi, and Pichincha

provinces (Zahlbruckner 1905, 1907; Arvidsson, 1991, Sklenář et al. 2010). Our collections constitute first records of *T. vermicularis* for Azuay and Loja provinces.

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Three lichen species in *Buellia*, *Catillaria*, and *Cheiromycina*, new to Poland

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Abstract—Three sterile crustose lichen species, *Buellia arborea* from the Tatra Mts and *Catillaria fungoides* and *Cheiromycina reimeri* from Białowieża National Park, are recorded as new to Poland. The record of *Cheiromycina reimeri* is also the first from Europe. Characteristics of all three species, notes on similar taxa, distribution, and habitat preferences are provided.

Key words—sorediate lichens, lichenized fungi, *Ascomycota*

Introduction

Crustose, persistently or usually sterile lichens have been for a long time one of the most neglected groups of species in the Polish lichen biota, especially before thin layer chromatography (TLC) became a routinely used method in their determination. However, the knowledge on their occurrences, distribution, and ecology have been much improved in the past 15 years, including species reported as either new to Poland or new to science (e.g., Kukwa & Kubiak 2007, Czarnota et al. 2009, Kukwa & Jabłońska 2009, Czarnota & Guzow-Krzemińska 2010, Kukwa et al. 2012, Zduńczyk & Kukwa 2014, Guzow-Krzemińska et al. 2016).

In this paper, we present the first Polish records of three crustose lichen species: *Buellia arborea*, *Catillaria fungoides*, and *Cheiromycina reimeri*.

Material & methods

The material studied is deposited in following herbaria: GPN, FR, KTC, and UGDA. The characteristics of taxa are based on Polish specimens, if not otherwise stated. Lichen substances were studied by thin-layer chromatography (TLC) using the methods of Orange et al. (2001). Reactions of the thalli with C (commercial bleach containing sodium hypochlorite), K (10% solution of potassium hydroxide), and Pd (alcohol solution of paraphenylenediamine) were also checked.

The localities examined are presented according to the modified ATPOL grid square system (Kukwa et al. 2010, 2013 and literature cited therein). The numeration of collecting plots in the Białowieża National Park follows Faliński & Mułenko (1997).

The species

Buellia arborea Coppins & Tønsberg, Sommerfeltia 14: 111. 1992.

Description—Thallus inconspicuous, white, endoxylic except for the concave to convex, crateriform, punctiform to slightly elongated soralia producing mostly dull, blackish-green pigmented soredia. Soredia single or loosely aggregated, externally encrusted with crystals, which more or less dissolve in K, composed of several algal cells surrounded by a stout, colorless or greyish-green pigmented hyphal layer. Apothecia absent. Non-pigmented soredia K+ yellowish, Pd–, C+ yellowish; pigment in soredial walls K+ dulling; atranorin and placodiolic acid detected by TLC.

Specimen examined—**POLAND. Tatra Mts, High Tatra Mts**, Tatra National Park, forest unit no 53f, Dolina Rybiego Potoku valley, below Opalone Mt., 49°13′08.9″N 20°05′05.6″E, alt. 1330 m, ATPOL grid square Ge–60, *Plagiothecio-Piceetum*, on wood of decaying log (*Picea abies*), 2.07.2010, P. Czarnota 6700 (GPN).

Distribution & habitat—Although *Buellia arborea* is widespread in the Holarctic, it is a rarely reported lichen, most probably due to its inconspicuous thalli, which are usually sterile. It has been reported from Europe (Estonia, Germany, Great Britain, Norway, Russia: Ural Mts, Slovakia, Sweden, Switzerland, Ukraine), Asia (Russia: Siberia) and North America (Canada, USA) (Dietrich et al. 1992, Tønsberg 1992, Foucard & Nordin 1999, Tønsberg & McCune 2001, Bogdan 2002, Swensson et al. 2004, Lisická 2005, Brackel & Kocourková 2006, Elvebakk & Bjerke 2006, Spribille & Björk 2008, Coppins et al. 2009, Suija et al. 2009, Urbanavichus & Andreev 2010, Davydov & Printzen 2012, Wirth et al. 2013).

In Poland *B. arborea* was found on the exposed wood of a spruce log in the upper montane Carpathian spruce forest.

Comments—The characteristic of Polish material of *B. arborea* agrees with the species description in Tønsberg (1992). For a description of apothecia, which were absent in the examined specimen, see Tønsberg (1992).

Buellia arborea resembles three other lichens—*B. griseovirens* (Turner & Borrer ex Sm.) Almb., *Violella fucata* (Stirt.) T. Sprib., and *Xylographa vitiligo* (Ach.) J.R. Laundon—all of which can also form crateriform soralia with dark-colored soredia and may occupy the same habitat (decorticated logs). All these species occur in Poland and differ in their secondary metabolite compositions: *B. arborea* produces placodiolic acid and does not react with Pd, whereas *B. griseovirens* contains norstictic acid (Pd+ yellow to orange); *V. fucata* has fumarprotocetraric acid (Pd+ red); and *X. vitiligo* produces stictic acid (Pd+ orange) (Tønsberg 1992, Wirth et al. 2013).

Lecanora orae-frigidae R. Sant. is another epixylic species with delimited soralia producing dark outer soredia. It produces, however, usnic acid, zeorin, and xanthones and occurs along the seashores in the arctic and boreal regions of the Northern Hemisphere (Brodo & Vänskä 1984).

Catillaria fungoides Etayo & van den Boom, Lichenologist 33: 107. 2001.

Description—Thallus thin, not clearly delimited, whitish. Soralia ≤0.25 mm in diam., dark brown to black, slightly convex, discrete or sometimes confluent. Soredia globose, farinose, ≤20 µm in diam., the outermost dark brown to almost black, but inner soredia hyaline with colorless or pale brown walls. Apothecia absent. Soredia C–, K– (blood-red crystals not formed in squash-preparation) and Pd–; pigment in soredial walls also K negative; material too scanty for TLC.

Specimen examined—**POLAND. North Podlasie District, Bielska Plain**, Białowieża National Park, forest unit no 256, plot L06, 52°46′15″N 23°52′04″E, ATPOL grid square Cg–55, *Circaeo-Alnetum*, on the bark of a hardwood log (*Fraxinus excelsior*), 19.08.2015, M. Kukwa 17314a, A. Łubek (KTC, UGDA).

Distribution & habitat—*Catillaria fungoides* is an epiphytic lichen reported from Africa (Cape Verde), Asia (South Korea, Turkey) and Europe (Czech Republic, the Netherlands, Portugal, Slovakia, Spain) (Boom & Etayo 2001, Boom et al. 2007, Malíček et al. 2014, Aptroot & Moon 2015).

In Poland *C. fungoides* was found on the bark of a hardwood log in a humid, hardwood stand in the Białowieża Forest. The species was sought by the first authors in northern Poland several times; however, it could not be found there. This suggests it is a rare lichen, perhaps confined to well-preserved forest habitats.

Comments—The characteristics of Polish material of *C. fungoides* agree with the species as described in Boom & Etayo (2001). For the description of apothecia, which were absent in the examined specimen, see Boom & Etayo (2001).

Catillaria fungoides is characterized by dark brown to black soralia (resembling sporodochia of some fungi) and the absence of lichen metabolites detectable by standard TLC. This species can easily be confused with *Rimularia fuscosora* Muhr & Tønsberg, which has larger (≤0.6 mm diam.) soralia and contains a trace of norstictic acid (Tønsberg 1992, Boom & Etayo 2001). Norstictic acid can be difficult to detect in TLC when it occurs in trace amounts, but blood-red crystals indicative of its presence are formed in squash-preparations of soralia in K (Tønsberg 1992).

Dark soredia are also formed by *Buellia griseovirens* and *Placynthiella dasaea* (Stirt.) Tønsberg, but they are never as dark brown or black as in *C. fungoides*. Additionally, all three species differ in their chemistries: *C. fungoides* lacks lichen metabolites detectable by TLC; *B. griseovirens* produces atranorin and norstictic acid (sometimes only atranorin or only norstictic acid); and *P. dasaea* contains gyrophoric and lecanoric acids (soredia C+ red) (Tønsberg 1992, Boom & Etayo 2001).

Cheiromycina reimeri Printzen, Nova Hedwigia 84: 262. 2007.

Description—Thallus thin, crustose, contiguous, smooth, greyish green, matt. Conidiomata sporodochia, white, greyish-white or grey, discrete, single or confluent, more or less ellipsoid, up to 0.80 × 0.50 mm, initially flat to moderately convex. Conidiogenous cells terminal or lateral, globose or broadly ellipsoidal, 7–10 × 7–8 µm. Conidia palmate, 2–4 times dichotomously branched, 20–30 µm wide, 15–25 µm tall, multi-septate, terminal branches with 1–2 cells.

Specimens examined—**POLAND. North Podlasie District, Bielska Plain**, Białowieża National Park, forest unit no 256, plot H10, 52°46′05″N 23°52′25″E, ATPOL grid square Cg–55, *Tilio-Carpinetum*, on the bark of *Carpinus betulus*, 6.10.2015, M. Kukwa 17681, A. Łubek (KTC, UGDA); plot I09, 52°46′08″N 23°52′20″E, *Tilio-Carpinetum*, on bark of *Carpinus betulus*, 23.08.2015, M. Kukwa 17422, A. Łubek (FR, KTC, UGDA).

Distribution & habitat—*Cheiromycina reimeri* is a corticolous lichen that has been known previously from Turkey and the Russian Far East (Sakhalin Island) (Printzen 2007).

In Poland *C. reimeri* has been found only in Białowieża National Park, twice on the bark of *Carpinus betulus*. Our collections represent the first records of the species in Europe.

Comments—The characteristics of Polish material of *C. reimeri* agrees with the species as described in Printzen (2007).

Cheiromycina reimeri can be confused with two other *Cheiromycina* species: *C. flabelliformis* B. Sutton and *C. petri* D. Hawksw. & Poelt. Both, *C. flabelliformis*

and *C. reimeri* have conidiogenous cells that are distinctly larger than the conidial cells, but in *C. flabelliformis*, the conidiogenous cells do not exceed 9 µm, whereas in *C. reimeri* they are consistently larger than 9 µm. Additionally, the terminal branches of the conidia in *C. flabelliformis* comprise up to 5 cells, while in *C. reimeri* they are 1–3-septate (Printzen 2007). *Cheiromycina petri* can be distinguished from *C. reimeri* by its conidiogenous cells, which are not (or only slightly) larger than its conidial cells, whereas in *C. reimeri*, the conidiogenous cells are always conspicuously larger (Printzen 2007).

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Additions to the mycobiota of Poland

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Abstract—One saprobic fungus (*Agyrium rufum*), one facultative lichenicolous fungus (*Trimmatostroma quercicola*), and six obligatory lichenicolous fungi (*Arthonia coronata*, *Cornutispora intermedia*, *Didymocyrtis melanelixiae*, *Minutoexcipula mariana*, *Stigmidium rivulorum*, and *Weddellomyces xanthoparmeliae*) are reported as new to Poland. *Lecanora pulicaris* is a new host for *Cornutispora intermedia* and *Ochrolechia turneri* for *Minutoexcipula mariana*. Seven of the reported species were found in natural forest communities in Białowieża National Park. Discussions on characteristics of each species and distributions are also provided.

Key words—mitosporic fungi, *Ascomycota*

Introduction

Lichens, lichenicolous fungi, and allied fungi are a reasonably well-known group of organisms in Poland (Fałtynowicz 2003, Czyżewska & Kukwa 2009). However, additional research, especially in areas with well-preserved natural forests, continue to provide new data on their occurrence, including the discovery of species new to science (Kukwa & Diederich 2005, Kukwa & Kubiak 2007, Kukwa & Flakus 2009, Zhurbenko et al 2009, Kukwa et al. 2010, 2012, 2013, Guzow-Krzemińska et al. 2016). Here we present the first Polish records of eight species of saprobic and lichenicolous fungi.

Material & methods

The material studied is deposited in KTC, LBL, and UGDA. The technical descriptions are based on Polish material, except for *Didymocyrtis melanelixiae,*

where the teleomorph was not found among our samples. The localities are presented according to the modified ATPOL grid square system (Cieśliński & Fałtynowicz 1993, Kukwa et al. 2002). For species collected in Białowieża National Park, the numeration of collecting plots follows Faliński & Mułenko (1997).

The species

Agyrium rufum (Pers.) Fr., Syst. Mycol. 2: 232. 1822.

Thallus endoxylic, non-lichenized, visible as paler patches of the substratum with scattered, minute, flat to convex, irregularly roundish, pale orange to red-brown apothecia. Exciple soon excluded, but visible in young apothecia, consisting of branched and anastomosing hyphae, pale to deep orange-brown in section, K+ orange-red. Hymenium 70–80 µm thick, intensely I+ blue, K+ orange-red. Paraphyses branched and anastomosing. Asci broadly clavate, of *Trapelia*-type, 8-spored. Ascospores simple, hyaline, 10–12 × (4.5–)5–5.5 µm.

Ecology—In Poland *A. rufum* was found only in Białowieża National Park (NE Poland) in two localities in natural pine-oak mixed forest growing together with *Micarea soralifera* Guz.-Krzemiń. et al. and other *Micarea* species on decorticated logs. It is perhaps more common in Poland, but due to the small size of apothecia easily overlooked in the field.

Specimens examined—**POLAND. North Podlasie District**, Bielska Plain, Białowieża National Park, forest unit no 256, plot G03, 52°46′02″N 23°51′48″E, ATPOL grid square Cg–55, *Pino-Quercetum*, on decorticated log, 05.2015, M. Kukwa 15930, A. Łubek (KTC, UGDA); plot G04, 52°46′02″N 23°51′53″E, *Pino-Quercetum*, on decorticated log, 05.2015, M. Kukwa 15939, A. Łubek (KTC).

Comments— For a detailed description see also Dobson et al. (2009).

This is a non-lichenized, saprobic fungus that is superficially similar to *Micarea* species with dark apothecia (e.g., *M. denigrata* (Fr.) Hedl. and *M. misella* (Nyl.) Hedl.). *Agyrium rufum* is readily distinguished by its *Trapelia*-type asci and the lack of photobiont in the thallus (Dobson et al. 2009).

Molecular phylogenetic analyses have shown that *A. rufum* is closely related to *Pertusaria* DC. s. str., from which it is easily morphologically distinguished by its much smaller apothecia and *Trapelia*-type asci (Hodkinson & Lendemer 2011).

Agyrium rufum has been found in many European countries growing on wood of diverse woody plants in various forest ecosystems (e.g., Coppins & Coppins 2006, Dobson et al. 2009, Mihál et al. 2012, Malíček & Palice 2013, Spribille et al. 2014). The species has also been found in Australia (Kantvilas 2002).

Arthonia coronata Etayo, Bull. Soc. Linn. Provence 47: 95. 1996.

Ascomata formed on discoloured parts of the host thalli, black, convex, usually covered with simple, dark, <25 µm long hairs giving the surface a roughened appearance. Hymenium and hypothecium hyaline. Ascospores 1-septate, 10–13 × 3.5–4 µm, hyaline at first, becoming pale brown with age.

Ecology—In Poland *A. coronata* was recorded in Białowieża National Park where it is found on podetia of sorediate *Cladonia* species growing on wood and bark of logs and stumps in pine-oak mixed forest and fresh (spruce)-pine forest.

Specimens examined—**POLAND. North Podlasie District**, Bielsk Plain, Białowieża National Park, ATPOL grid square Cg–55, forest unit no 256, plot E09, 52°45′55"N 23°52′20"E, *Pino-Quercetum*, on *Cladonia subulata*, on stump, 08.2015, A. Łubek (KTC); plot G01, 52°46′02″N 23°51′37″E, *Pino-Quercetum*, on *C. coniocraea* growing on stump, 05.2015, M. Kukwa 15918, A. Łubek (KTC); plot G04, 52°46′02″N 23°51′53″E, *Pino-Quercetum*, on *C. grayi* growing on bark of log, 05.2015, M. Kukwa 15897, A. Łubek (KTC) and *C. digitata* growing on decorticated log, 05.2015, A. Łubek (KTC); plot H04, 52°46′05″N 23°51′53″E *Peucedano-Pinetum*, on *C. grayi* and *C. coniocraea* growing on decorticated log, 05.2014, M. Kukwa 13592, A. Łubek (KTC, UGDA); plot L07, *Querco-Piceetum*, on *C. grayi* growing on decorticated log, 29.09.2015, M. Kukwa 17540a, A Łubek (UGDA); plot M01, 52°46′18″N 23°51′37″E, *Peucedano-Pinetum*, on *C. grayi* growing on wood of log, 05.2015, A. Łubek (KTC).

Comments—For descriptions see also Etayo (1996) and Coppins & Aptroot (2009).

Arthonia coronata is distinguished by its black and convex ascomata with usually simple dark hairs (best visible in apothecial section under the microscope) giving the ascomata a roughened appearance, and the 1-septate hyaline ascospores that become pale brown with age, ascospores. It cannot be with confused with any other lichenicolous fungi inhabiting *Cladonia* thalli (Etayo 1996).

The species was first found in France and Spain on soralia of *Flavoparmelia caperata* (L.) Hale but later was also recorded from other countries on thalli of *Cladonia* species. The presence of the fungus is manifested by a discolouration of the host thallus by black ascomata (Etayo 1996, Coppins & Aptroot 2009, Diederich et al. 2012).

Arthonia coronata has been found in Africa (Canary Islands), North America, and Europe (Austria, Belarus, Belgium, France, Germany, Great Britain, Italy, Luxemburg, Portugal, Spain, Sweden) (Etayo 1996, Coppins & Aptroot 2009, Diederich et al. 2012, Boom 2013, Svensson et al. 2013, Roux et al. 2016, Tsurykau et al. 2016).

Cornutispora intermedia Punith. & D. Hawksw., Mycol. Res. 107: 920. 2003.

Anamorph—This species is known only in the anamorphic state, which causes discolourations of host thalli. Pycnidia scattered, immersed or with upper parts exposed over the host thallus, globose with pseudoparenchymatous walls, pale brown to dark brown, c. 150–160 µm in diam. Conidia holoblastic, hyaline, tri-radiate; main axes 9–12 µm high (from the truncate bases to a point where the conidia branch) and 2–2.5 µm wide; divergent arms 3–5 × 2–2.5 µm, with swollen bases. Both axes and arms end with narrow, 3–5 µm long, tubular structures or apical appendages.

Ecology—In Poland the species was found in one locality in Białowieża National Park on the thallus of *Lecanora pulicaris* (Pers.) Ach. in a pine-oak mixed forest; *L. pulicaris* is a new host lichen for this fungus.

Specimen examined—**POLAND. North Podlasie District**, Bielsk Plain, Białowieża Forest, Białowieża National Park, ATPOL grid square Cg–55, forest unit no 256, plot D03, *Pino-Quercetum*, on *Lecanora pulicaris* growing on bark of twigs of fallen *Picea abies*, May 2015, A. Łubek s.n. (KTC).

Comments—For a full description of *C. intermedia*, see also Punithalingam (2003).

Cornutispora intermedia is the third species of the genus reported from Poland, the others being *C. ciliata* Kalb and *C. lichenicola* D. Hawksw. & B. Sutton (Kukwa & Flakus 2009, Kukwa et al. 2013). In *C. ciliata* the main conidial axes are shorter (usually 6.5–9 µm long; in *C. intermedia* 8–11 µm) and the bases of the divergent arms are bulbous (only swollen in *C. intermedia*). *Cornutispora lichenicola* has narrower main conidial axes (<2 µm wide; in *C. intermedia* >2 µm) (Punithalingam 2003).

Cornutispora limaciformis Piroz. and *C. pittii* D. Hawksw. & Punith. produce conidia of very similar in shape to those in *C. intermedia*, but the main conidial axes are >13 µm long in *C. limaciformis* and <3.5 µm in *C. pittii*. In addition, *C. limaciformis* is a non-lichenicolous fungus growing on apothecia of *Therrya fuckelii* (Rehm) Kujala (Punithalingam 2003); neither of these species has yet been found in Poland.

Cornutispora intermedia has also been reported from Europe (Germany, Italy, Peru) and USA on thalli of *Lecanora chlarotera* Nyl., *Ochrolechia* sp., *Phaeophyscia orbicularis* (Neck.) Moberg, *Physcia* sp., *Thamnolia vermicularis* (Sw.) Schaer., *Xanthoria parietina* (L.) Th. Fr., and *X. polycarpa* (Hoffm.) Rieber (Punithalingam 2003, Brackel 2009, 2014, Etayo 2010, Schiefelbein et al. 2014).

Didymocyrtis melanelixiae (Brackel) Diederich, Harris & Etayo, Fungal Div. 74: 74. 2015.

Anamorph causing discolourations of the host thalli. Pycnidia immersed in the host thallus, <90 µm diam., macroscopically black; walls pseudoparenchymatous, brown (darker around ostiola). Conidia broadly ellipsoidal, rounded at both ends, smooth-walled, hyaline, with one large (rarely two smaller) guttule, 4.5–5 × 3.5 µm. Sexual stage not found.

Ecology—In Poland the species has been found only at one locality in southern Poland on thalli of *Parmelia sulcata* Taylor.

Specimens examined—**POLAND.** Roztocze Środkowe, Roztoczański National Park, Florianka settlement, 50°33′25"N 22°59′15"E, alt. c. 250 m, ATPOL grid square Fg–01, on *Parmelia sulcata* growing on wooden fence, 17.09.2015, M. Kukwa 17532 (LBL, UGDA).

Comments—Only the anamorphic state was seen in the Polish material; according to Ertz et al. (2015) ascomata are perithecial, the hamathecium consists of paraphysoids, and asci are 8-spored. Ascospores are usually 2-septate, slightly constricted at the septa, brown, verruculose, 11.5–15 × 4–5.5 µm. For full descriptions see also Brackel (2011) and Ertz et al. (2015).

Didymocyrtis melanelixiae in its anamorphic state can be confused with four other species producing simple hyaline conidia and growing on lichens of *Parmeliaceae*. The most common species, *Briancoppinsia cytospora* (Vouaux) Diederich et al., differs in its larger conidia (5–7 × 1.5–2 µm). *Phoma melanohaleicola* D. Hawksw. & Earl.-Benn. develops pycnidia with seta-like hyphae rising from the walls and does not damage the host thallus. In *P. everniae* D. Hawksw. the conidia measure 4.5–5.5 × 1–1.5 µm, whereas in *P. dubia* (Linds.) Sacc. & Trotter they are 3.5–5 × 1.5–2 µm (Hawksworth & Cole 2004, Brackel 2011). Neither *P. everniae* nor *P. dubia* has been found in Poland.

Didymocyrtis melanelixiae is widespread, being recorded from Europe (Austria, Belgium, France, Great Britain, Italy, Spain, Switzerland), North America (Canada, USA), South America (Ecuador), and La Reunion, always on members of *Parmeliaceae* Eschw. (Brackel 2011, Ertz et al. 2015, Hafellner 2015).

Minutoexcipula mariana V. Atienza, Biblioth. Lichenol. 82: 142. 2002.

Conidiomata sporodochia-like, concave at first, but later becoming confluent and convoluted. Exciple distinct, thin, pseudoparenchymatic. Conidiophores mostly unbranched, macronematous, conidiogenous cells almost hyaline, 5–6 × 1.5–2 µm with ≤3 annellations. Conidia 6–7 µm long, brown, 1-septate, holoblastic, acrogenous, smooth-walled, with truncate bases.

Ecology—Collected from the thallus of *Ochrolechia turneri* (Sm.) Hasselrot growing on a fallen tree in a humid streamside within a black alder-ash forest in Białowieża National Park. It is the first record of *Minutoexcipula mariana* on an *Ochrolechia* species. As *Lepra*, *Ochrolechia*, and *Pertusaria* are very closely related (Schmitt & Lumbsch 2004), we consider this host range extension of understandable.

Specimens examined—**POLAND. North Podlasie District**, Bielsk Plain, Białowieża National Park, forest unit no 256, plot O02, 52°46′24″N 23°51′42″E, ATPOL grid square Cg–55, *Circaeo-Alnetum*, on *Ochrolechia turneri* growing on bark of fallen *Fraxinus excelsior*, 10.2014, M. Kukwa 13086, A Łubek (KTC, UGDA).

Comments—For descriptions see also Atienza (2002) and Diederich (2003).

Minutoexcipula mariana can be confused with two other species growing on members of *Pertusariales: M. tuckerae* V. Atienza & D. Hawksw. and *M. tuerkii* Hafellner. *Minutoexcipula tuckerae* differs in its convex conidiomata and 2–3-branched conidiophores, whereas *M. tuerkii* has larger conidia and does not develop conidiophores (Atienza 2002, Diederich 2003). *Minutoexcipula tuerkii* was reported from Poland by Kukwa & Flakus (2009).

Two *Lichenodiplis* species, *L. pertusariicola* (Nyl.) Diederich and *L. hawksworthii* F. Berger & Diederich, are also similar, but both produce closed conidiomata; additionally *L. pertusariicola* has aseptate conidia whereas *L. hawksworthii* has larger conidia (8.5–13 × 5.5–5.5 µm) (Diederich 2003). Of these two, only *L. pertusariicola* has been recorded in Poland (Czyżewska & Kukwa 2009).

Minutoexcipula mariana has previously been recorded in Asia (Japan, as *M.* cf. *mariana*), Europe (Germany, Iceland, Italy, Portugal Spain, the Azores), North America (Canada, USA, Mexico), and New Caledonia and only on *Lepra* and *Pertusaria* species: *L. albescens* (Huds.) Hafellner, *P. glomerata* (Ach.) Schaer., *P. heterochroa* (Müll. Arg.) Erichsen, *P. pluripuncta* Nyl., *P. xanthodes* Müll. Arg., and unidentified *Pertusaria* sp. (Atienza 2002, Diederich 2003, Zhurbenko 2013, Brackel 2014, Zhurbenko et al. 2015).

Stigmidium rivulorum (Kernst.) Cl. Roux & Nav.-Ros.,
Bull. Soc. Linn. Provence 44: 449. 1994.

Ascomata numerous, ≤65 µm diam., globose to subglobose. Paraphyses absent; periphyses present around ostiola. Asci 8-spored. Ascospores 1-septate, hyaline, upper cell slightly wider and shorter than lower, 13.5–15 × 4–5 µm.

Ecology—Collected in a small water spring in a shaded forest in northern Poland. The species is perhaps more widespread, but due to the small dimensions of perithecia it is easily overlooked.

Specimen examined—**POLAND. Gdańsk Pomerania**, Wysoczyzna Żarnowiecka, Pużyckie Łęgi nature reserve, 54°38′N 17°51′E, ATPOL grid square A3c–54, a small water spring in *Circaeo-Alnetum*, on *Verrucaria hydrophila* growing on pebble, 12.08.2015, M. Kukwa 17069b (UGDA).

Comments—For full descriptions, consult Roux & Navarro-Rosinés (1994), Molitor & Diederich (1997), and Zhurbenko & Hafellner (1999).

Stigmidium rivulorum is confined to freshwater *Verrucaria* and *Staurothele* species. Three other *Stigmidium* species also grow on *Verrucariaceae* Eschw.: *S. clauzadei* Cl. Roux & Nav.-Ros. has larger ascomata (80–130 × 65–120 µm) and wider ascospores (5–6.5 µm) and grows on non-aquatic *Verrucaria* species (Roux & Navarro-Rosinés 1994); *S. marinum* (Deakin) Swinscow is parasitic on marine *Verrucaria* growing on seashores and produces larger ascomata (Swinscow 1965); and *S. tetrasporum* Etayo has 4-spored asci and wider ascomata (80–150 µm diam.), and was found on a sterile thallus probably belonging to *Verrucariaceae* (Etayo 1994). Previously only *S. clauzadei* had been found in Poland (Czyżewska & Kukwa 2009).

Stigmidium rivulorum has been found in Europe (Austria, Denmark: Faroe Islands, Germany, Great Britain, Italy, Luxembourg) and Asia (Russia: Siberia, South Korea) on thalli of *Verrucaria aquatilis* Mudd and other freshwater species of *Verrucaria* Schrad. and *Staurothele* Norm. (Alstrup et al. 1994, Molitor & Diederich 1997, Coppins & Coppins 1999, Zhurbenko & Hafellner 1999, Thüs & Dornes 2003, Orange et al. 2009, Brackel 2014, Berger & Türk 2015, Kondratyuk et al. 2015).

Trimmatostroma quercicola Diederich, U. Braun & Heuchert, Bull. Soc. Nat. Luxemb. 111: 52. 2010.

Hyphomycete facultative lichenicolous, growing on oak bark, often overgrowing degenerate crustose lichen thalli or corticolous algae. Colonies an irregular net of blackish hyphae that are effuse, loose to dense, black, formed by short, often agglomerated, irregular and branched conidial chains. Conidia in simple or branched chains, globose to oblong, ≤4-septate (usually 3-septate), reddish-brown in H_2O, olivaceous-brown in K, ≤10 µm wide, with <2 µm thick, rugose-rimulose to verrucose walls.

Ecology—In Poland *Trimmatostroma quercicola* was found on the bark of old oak in moist oak-spruce forest in Białowieża National Park. It is probably widespread but overlooked due to the inconspicuous thalli.

Specimens examined—**POLAND. North Podlasie District**, Bielsk Plain, Białowieża National Park, forest unit no 256, plot E07, 52°45′55″N 23°52′10″E, ATPOL grid square Cg–55, *Querco-Piceetum*, on degenerated lichen thalli growing on bark of *Quercus robur*, 08.2014, M. Kukwa 14117 (KTC, UGDA).

Comments—For a complete description see also Diederich et al. (2010).

In general *T. quercicola* produces much thicker conidial chains than the other *Trimmatostroma* species, which are easily visible under stereomicroscope as an irregular blackish hyphal net. It also differs from *T. lecanoricola* Diederich et al. by its orange-brown, K+ olivaceous conidia, and from *T. dendrographae* Diederich et al. in producing brown conidia (without a distinct olivaceous tinge). In *T. hierrense* Diederich & Ertz the conidia often have darker outer walls on one side, whereas in *T. glebarum* Brackel conidia are 0–pluri-septate with cracking outermost wall layers, and colonies formed of hyphae developing a visible dark net on or near the surface of *Flavoparmelia caperata* thallus (Diederich et al. 2010, Brackel 2015). *Trimmatostroma cetrariae* Brackel has usually 1-septate conidia (Zhurbenko & Brackel 2013), *T. umbilicariicola* Heuchert & U. Braun develops multicellular and muriform segments in the fertile threads, and conidia of *T. varicellariae* Heuchert & U. Braun are dark brown to black (colour not changing in K) (Heuchert & Braun 2014).

The habit of *T. quercicola* (and also other lichenicolous *Trimmatostroma* species) is similar to *Intralichen* spp., which differ by having smooth-walled, paler, mostly 0–2-septate conidia and conidiophores usually that are completely immersed in the host thalli or apothecia (Diederich et al. 2010). *Taeniolella* species can be distinguished by conidia formed in acropetal chains and semi-macronematous, superficial conidiophores; multicellular aggregations of conidial cells are also absent in *Taeniolella* but are common in *Trimmatostroma* species (Diederich et al. 2010).

This recently described species is known from Luxembourg and Belgium (Diederich et al. 2012).

Weddellomyces xanthoparmeliae Calat. & Nav.-Ros., Mycotaxon 69: 505. 1998.

Ascomata black, perithecioid, scattered in the host thallus, which is discoloured where infected. Perithecia arising singly or in small groups, at first immersed, but later becoming partly exposed over the host thallus. Ascomatal wall formed of cephalothecioid plates. Asci 8-spored. Hamathecium of branched and anastomosing hyphae, ascospores are (1–)3-septate, brown, finely granulose, 25–30 × 8–10 µm.

Ecology—Found on the thallus of *X. conspersa* in the Sudety Mts, Poland.

Specimen examined—**POLAND. Sudety Mts**, Góry Sowie mts, near Jugów village, 50°38′51″N 16°30′19″E, elev. 720 m, ATPOL grid square Eb–95, on *Xanthoparmelia conspersa* growing on rock, 08.2015, E. Ossowska s.n. (UGDA).

Comments—For other descriptions see also Calatayud & Navarro-Rosinés (1998) and Kocourková (1999).

Weddellomyces xanthoparmeliae is easily recognizable by its formation of orange necrotic spots on the host thalli, an ascomatal wall formed of cephalothecioid plates, usually 8-spored asci, and brown finely granulose (1–)3-septate ascospores measuring 25–30 × 8–10 µm. It differs from all other *Weddellomyces* species in its smaller ascospores and host selection (Calatayud & Navarro-Rosinés 1998). Other lichenicolous fungi with closed ascomata growing on *Xanthoparmelia* species include *Stigmidium neofusceliae* Calat. & Triebel, *S. xanthoparmeliarum* Hafellner, and *Lichenostigma cosmopolites* Hafellner & Calat. (all distinguished by 1-septate ascospores), and *Roselliniella atlantica* Matzer & Hafellner, which produces larger and usually aseptate ascospores (Matzer & Hafellner 1990, Hafellner 1994, Calatayud & Triebel 1999, Hafellner & Calatayud 1999).

So far *W. xanthoparmeliae* is known from Europe (Czech Republic, Germany, Spain) and North America. Hosts are usually *Xanthoparmelia* species containing usnic acid: *X. conspersa* (Ehrh. ex Ach.) Hale, *X. mexicana* (Gyeln.) Hale, *X.* cf. *protomatrae* (Gyeln.) Hale, and *X. somloensis* (Gyeln.) Hale (Calatayud & Navarro-Rosinés 1998, Kocourková 1999, Brackel 2007, Kocourková & Knudsen 2011), but Brackel (2014) reported it from thalli of brown *X. verruculifera* (Nyl.) O. Blanco et al.

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Macrocyclic *Edythea quitensis* rust on *Berberis hallii* in Ecuador

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Abstract—The full macrocyclic autoecious life cycle of five spore stages was confirmed for *Edythea quitensis* on *Berberis hallii* in the highlands of Ecuador. The spermogonial and aecial stages had not previously been recorded. Spore descriptions and DNA sequence analysis are included.

Key words—*Pucciniales,* phylogeny, taxonomy

Introduction

Reports of rust occurring on *Berberis* in Ecuador date from 1891, when Lagerheim collected and distributed specimens of two species that he labeled as "*Sphenospora quitensis*" and "*Diorchidium berberidis*"; subsequently, Arthur (1918) published descriptions of these specimens as *Uropyxis quitensis* and *Sphenospora berberidis*. Jackson (1931) proposed a new genus *Edythea* to accommodate three South American species of rust on *Berberis:* the two collected by Lagerheim (recombined as *E. quitensis* [the type species] and *E. berberidis* (Lagerh. ex Arthur) H.S. Jacks.) and a new species from Bolivia (*E. tenella* H.S. Jacks. & Holw.). Jackson (1931) depicts *Edythea* as having urediniospores and teliospores "borne in a very characteristic and presumably unusual manner" because of the lack of proper sori, as the mycelial threads emerge from the stomata without rupturing the leaf tissue to give rise to spores. The characteristics described by Jackson (1931) to

differentiate between the three species of *Edythea* were the presence of a vertical septum on teliospores for *E. berberidis,* a variable septum with nearly globoid teliospores for *E. quitensis,* and a variable septum but with ellipsoid teliospores for *E. tenella.* Urediniospores from the three species are described as quite similar, but there is no mention of any spore stage, other than urediniospores or teliospores, for the three *Edythea* species described (Jackson 1931).

The host family of these rusts, the *Berberidaceae,* includes 13 genera, of which only the genus *Berberis* L. is present in Ecuador. With over 500 species worldwide, *Berberis* (including *Mahonia* Nutt.) is the largest genus of the family. There are two important centers of diversity for *Berberis,* one in Eurasia with around 300 species mostly in China and the Himalayas and another in South America with approximately 200 species (Ahrendt 1961). The simple leaf type occurs throughout South America, while the compound leaf type (previously referred to *Mahonia*) does not reach South America, and is found only from Mexico to Costa Rica (Ulloa 2013). In Ecuador, 32 *Berberis* species have been reported, of which 16 are classified as endemic (Ulloa 2013). All species occur in the highlands over 2000 meters above sea level. The *Berberis* plant is used locally for coal, fencing, and firewood, with the fruit used in jams and the roots used for medicinal purposes and staining (Ulloa 2013).

In this paper we report the rust fungus *Edythea quitensis* found on *Berberis hallii* Hieron. in Ecuador, as having a full macrocyclic autoecious life cycle; sequence analysis of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA for three spore types also illustrates the phylogenetic relationship between the *Edythea* rust samples collected on *B. hallii* in Ecuador with rust collected from other *Berberis* species elsewhere.

Materials & methods

Collection and spore morphology

Rust infected leaves were collected from *Berberis hallii* in Quito, Pichincha province. Fresh spores were examined microscopically in water, and teliospores, urediniospores, and aeciospores were measured using Infinity Analysis software (Lumenera Corp., ON, Canada). Twenty random spores from each collection were measured for each spore type. Germinating teliospores, basidiospores, and spermogonia were also observed. Collections were deposited in the QCAM fungarium at Pontifica Universidad Católica del Ecuador, Quito, Ecuador (Table 1).

Molecular analysis

DNA was extracted separately from aecia/spermogonia and uredinia/telia found on different collections of *B. hallii* leaves. The tissue was macerated with zirconium

Table 1. *Edythea quitensis* samples analyzed

Specimen	Geographical coordinates	Altitude [m a.s.l.]	Spore type observed*	GenBank no.
QCAM6449	–0.18646, –78.46178	2890	II, III	KX298148
QCAM6450	–0.17609, –78.46702	2917	0, I, II, III, IV	KX298149
QCAM6451	–0.17455, –78.46968	2879	II, III	KX298150
QCAM6452	–0.17711, –78.46658	2919	II, III	KX298151
QCAM6453	–0.17497, –78.46890	2890	II, III	KX298152
QCAM6454	–0.17708, –78.46670	2887	0, I, II, III, IV	KX298153

* Following Cummins & Hiratsuka 1983

Table 2. Rust sequences from GenBank included in the phylogenetic analysis

GenBank no.	Taxon	Country	Host	Reference
JX047488	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047486	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047489	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047484	*Puccinia* sp.	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047456	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047465	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047468	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047467	*P. graminis* f.sp. *tritici*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047477	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047478	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047482	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
JX047475	*P. graminis* f.sp. *avenae*	Sweden	*Berberis* sp.	Berlin et al. 2013
AY956558*	*P. striiformoides*	Iran	*Dactylis glomerata*	Abbasi et al. 2004
DQ417406*	*P. striiformis* f.sp. *tritici*	USA	*Triticum aestivum*	Barnes & Szabo 2007
GU382672	*P. striiformis* f.sp. *tritici*	USA	*Berberis chinensis*	Jin et al. 2010
GQ457304+	*P. pseudostriiformis*	USA	*Berberis* sp.	Jin et al. 2010
GQ457306+	*P. pseudostriiformis*	USA	*Berberis koreana*	Jin et al. 2010

* Best Blastn hits (from non-*Berberis* hosts) to *Edythea quitensis* ITS sequence
\+ Formerly *Puccinia striiformis* f.sp. *poae* (Jin et al. 2010)

beads and soaked in 0.5% SDS lysis buffer at 55°C for 3 hours. Protein precipitation was done with 4M guanidine isothyocianate solution, pH 7.5, followed by isopropanol and ethanol precipitation. The ITS (ITS1+5.8S+ITS2) region was amplified with primers ITS1F (Gardes & Bruns 1993) and ITS4BR (Vialle et al. 2009). The PCR cocktail comprised 0.2 µM of each primer, 1X PCR buffer, 3mM $MgCl_2$, 0.2mM dNTPs, 0.1 U/µL Platinum Taq DNA polymerase (Invitrogen, Brazil), and 1ng/µL of DNA. Amplification conditions were: 94°C for 3 min., 30 cycles of 94°C for 30 s, 50°C

for 30 s, and 72°C for 60 s, and a final extension at 72°C for 10 min. PCR products were sequenced at Macrogen Inc. (Seoul, South Korea). Sequences were edited in Geneious R9 (Kearse et al. 2012) and submitted to GenBank (http://www.ncbi.nlm.nih.gov). The alignments were deposited into TreeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S19447).

Sequence data were analyzed using Maximum Likelihood with a Custom (010212) Substitution model determined by jModelTest 2.0.2 (Darriba et al. 2012, Guindon & Gascuel 2003) according to Corrected Akaike Information Criterion (AICc). Sequence data available on GenBank from rust fungi infecting *Berberis* species from other countries were included in the analysis, as well as the two best Blastn hits (Table 2). A bootstrap of 1000 replicas was used.

Taxonomy

Edythea quitensis (Lagerh. ex Arthur) H.S. Jacks. & Holw., Mycologia 23: 99. 1931. Fig. 1

≡ *Uropyxis quitensis* Lagerh. ex Arthur, Bot. Gaz. 65: 464. 1918.

Type: Ecuador, Quito, stages II and III on *Berberis* sp., April 1891, G. Lagerheim (PUR 68850, holotype, originally deposited as "*Sphenospora quitensis*").

Spermogonia, aeciospores, urediniospores, teliospores, and basidiospores were observed on *B. hallii* in Quito. A total of six rust samples were analyzed, two showing all five spore stages and four only urediniospores and teliospores (Table 1). Spermogonia and aecia were found occurring simultaneously on leaves several times during the year, usually after rains.

Spermogonia were observed on the upper side of the barberry leaves, and aecial cups were present on the underside of the leaves. Spermogonia were yellow initially, becoming more orange in color as they aged. Aecial cups were bright orange. Aeciospores were ellipsoid to globose, 27.3–21.5 × 27.0–20.9 µm, verrucose, with a thin wall, yellow-orange in color. Urediniospores and teliospores were found year round and matched characteristics described by Jackson (1931); specifically: both spore types emerged through the stomates without rupturing the tissue and were cinnamon-brown in color; urediniospores were mostly globoid, single-celled, 29.5–22.8 × 28.2–22.6 µm; teliospores were globoid, two-celled, 27.6–22.0 × 31.6–24.0 µm with pedicels 21.4–30.0 × 5.5–6.6 µm. Germinating teliospores were observed forming a basidium with four basidiospores on sterigmata.

Phylogeny

ITS sequence analyses obtained separately for aecia/spermogonia and for uredinia/telia supported all within the same species (Fig. 2). The Ecuadorian samples were distinct from several rust species reported from *Berberis*

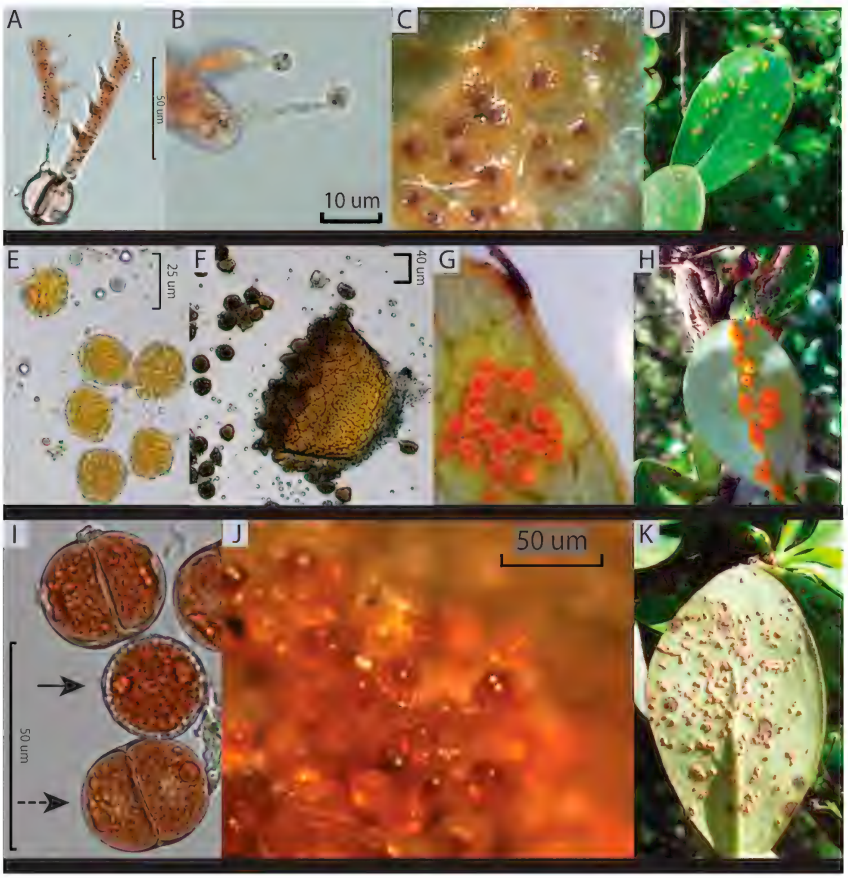

Fig. 1. *Edythea quitensis:* macrocyclic stages. A. basidia; B. basidiospores on sterigmata; C. spermogonia; D. spermogonial infection on upperside of barberry leaf; E. aeciospores; F. aecial cup; G. closeup of aecial cups on underside of barberry leaf; H. aecial infection on underside of barberry leaf; I. teliospore (dashed arrow) and urediniospore (whole arrow); J. mix of urediniospores and teliospores; K. urediniospore and teliospore infections on underside of barberry leaf.

from Sweden and the USA. The resulting topology was well supported by bootstrap values.

Discussion

The complete macrocyclic autoecious life cycle for *Edythea quitensis* occurring on *B. hallii* is described here for the first time. The three original

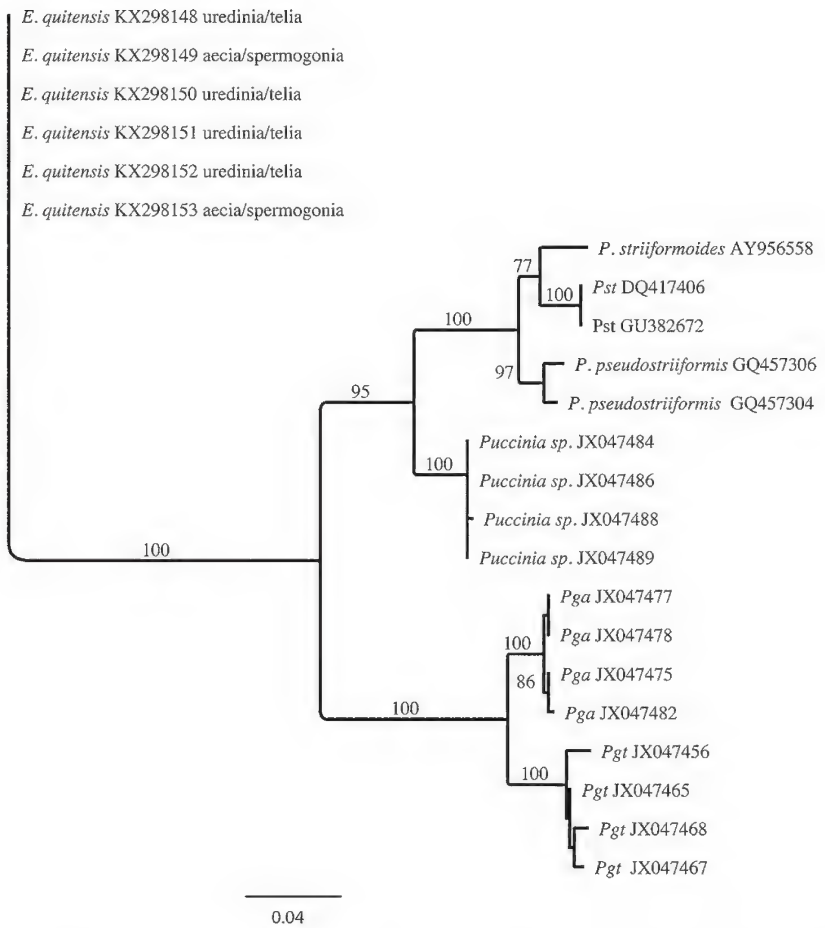

Fig. 2. Maximum Likelihood phylogenetic tree based on the ribosomal DNA sequence of the ITS1+5.8S+ITS2 region of *Edythea quitensis* and representative taxa of rust from *Berberis* spp., plus the two best Blastn hits not found on *Berberis* spp. One thousand bootstrap replicates were used; values ≥70% are given above branches.

Taxonomic abbreviations: *Pst* = *Puccinia striiformis* f.sp. *tritici; Pga* = *P. graminis* f.sp. *avenae; Pgt* = *P. graminis* f. sp. *tritici.*

Edythea rusts on *Berberis* and a fourth species, *E. soratensis* Ritschel (described from a 1920 collection on *Berberis* in Bolivia; Berndt et al. 2007), have not previously been subjected to repeated seasonal field observations. However, the increased attention being paid to *Berberis* spp. as potential

alternate hosts for stripe rust *Puccinia striiformis* Westend. in wheat growing areas (Jin et al. 2010, Zhao et al. 2013) afforded the opportunity for closer examination of the rust species occurring on *B. hallii* in Quito. It is likely that the original description of *E. quitensis* did not include the spermogonial and aecial stages due to the restricted time and number of collections that were available at that time.

The use of ITS sequence comparison of the different spore stages confirmed that the aeciospores, urediniospores, and teliospores analyzed belong to the same species.

Ecuador is within the center of diversity of *Berberis* species in South America, and more work is needed to describe the rust fungi that infect these hosts. Rust species description and identification are important for managing wheat rust resistance in Ecuador and adjacent regions as well as for documenting fungal biodiversity in these countries.

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Leucoagaricus ariminensis sp. nov., a lilac species from Italy

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Abstract—A new species, *Leucoagaricus ariminensis*, collected under *Cupressus sempervirens* in a park in Rimini (central Italy), is described and illustrated. The new species is compared with the other known lilac-coloured lepiotoid species, and its phylogenetic position, based on nrITS sequences, is determined. Colour photographs and illustrations of diagnostic anatomical characters are also provided.

Key words—*Agaricaceae*, *Agaricales*, lepiotaceous fungi, rDNA, taxonomy

Introduction

The genus *Leucoagaricus* Locq. ex Singer is considered to have a widespread geographical distribution even if it might appear more commonly linked to the tropical and sub-tropical zones of Africa and America (Singer 1986). Data in Vellinga (2004) indicate that the number of *Leucoagaricus* species in Europe increases as one moves southwards from the northern areas. Citations from the checklist of Italian fungi (Onofri et al. 2005) and other sources (Consiglio & Contu 2004, Zotti et al. 2008) establish occurrences of at least 52 *Leucoagaricus* species in Italy, a figure significantly higher than the numbers recorded in northern European areas (Vellinga 2004, Lange 2008). Of all species described in Italy, 58% occur in the north, 62% in the south (with islands included), and the majority, 73%, in the central regions. Considering only the non-

coastal northern regions, the number of species decreases to less than 40%, demonstrating the greater biodiversity of *Leucoagaricus* in milder climatic zones.

Numerous new species have been described in recent years (Ge 2010; Liang et al. 2010; Vellinga & Balsley 2010; Vellinga et al. 2010; Muñoz et al. 2012, 2014; Kumari & Atri 2013; Ye et al. 2014; Ge et al. 2015), a clear indication that the exact number of species in this genus is still incomplete. Some have been found in urban parks showing that these areas can be an interesting habitat for the study of this genus.

During a recent fungal biodiversity study conducted in Parco XXV Aprile, a public park in Rimini, a *Leucoagaricus* species was found, characterized by a dark violaceous pileus, violet/lilac tinged stipe and annulus rim, rather fleshy basidiomata, negative reaction to ammonia vapours, and white flesh that yellowed when handled. Comparison with the known *Leucoagaricus* species and ITS sequence analyses indicated that this species represents a novel taxon, which we describe below.

Materials & methods

Morphology

The macroscopic descriptions are based on observations of fresh material, which was photographed with a Canon EOS 60D camera fitted with a Canon EF-S 60 mm macro lens. The specimens were dried with an electric dryer. Voucher specimens were deposited in the Herbarium, Museo di Storia Naturale di Venezia, Venice, Italy (MCVE). The terminology used in the descriptions follows Vellinga (2001). Basidioma colours were coded according to Munsell (1994). The micro-morphological characters are based on the study of dried material rehydrated in KOH or NH_4OH; other reagents used were Congo Red for staining cell-walls, anionic phloxine for colouring the cytoplasm, Cresyl Blue for testing the metachromatic reaction of the spores, Melzer's reagent to detect spore dextrinoid reactions, glycerine tampon L4 for the observation of pigments, Chlorazol Black (L4C) for contrasting the septa. Spore nuclei were stained with DAPI (1 mg/mL) and observed with a fluorescence microscope. All observations and measurements were carried out with an optical trinocular Optrech B5 microscope, with ×10, ×25, ×40, ×60 lenses and a ×100 immersion lens. Microphotos were taken with a Canon EOS 60D camera connected to the microscope with an extension tube. At least 20 basidia, basidiospores, cystidia and terminal elements of the pileipellis were measured. Basidiospores were measured both from fresh spore deposit and from dried specimens. Basidiospore dimensions are expressed as (a) b–c (d), where (a) = minimum value, b = (average – standard deviation), c = (average + standard deviation), and (d) = maximum value. The following abbreviations are used: L = number of lamellae, and l = number of lamellulae; [x/y/z] indicates that altogether x spores, from y samples, from z collections were measured.

DNA extraction, PCR amplification, DNA sequencing

The DNA was extracted with NaOH (Osmundson et al. 2013); 2 mg of dry sample were homogenized in 250 µl of 0.5M NaOH with a pestle. After 10 minutes to allow for sedimentation, 5 µl of the extract was removed and diluted in 195 µl of 100mM Tris-HCl at pH 8.0, and 1 µl of the dilution was used as template DNA. The ITS region was amplified with primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR was performed in a 25 µl reaction volume following Gardes & Bruns (1993).

PCR products were purified and sequenced by IGA Technology Services (Udine, Italy).

Sequence alignment & phylogenetic analysis

Sequences were assembled and edited in Geneious v. 8.1.2 (Kearse et al. 2012) and then submitted to GenBank (see Fig. 1 for accession numbers).

The initial BLASTn results revealed that all reference sequences with identities >85% (and E = 0) to *L. ariminensis* belong to the Piloselli clade, following criteria reported in Vellinga (2010), Vellinga et al. (2010, 2011), and Muñoz et al. (2012, 2014).

Our dataset includes sequences in /Piloselli from previous phylogenetic studies (Vellinga 2010; Vellinga et al. 2010, 2011; Munoz et al. 2012, 2014) and other sequences with greatest similarity available in GenBank. We also included a sequence of *L. ionidicolor* (sequenced in this work, KU953373) collected in the Czech Republic and described in Holec (2009) and added for comparison six sequences representing *Leucoagaricus* sect. *Rubrotincti*. *Cystolepiota seminuda* was used as outgroup.

The sequences were aligned using MAFFT v 7.017 (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties.

Phylogenies were inferred with RAxML v.7.2.8. (Stamatakis 2006) and MrBayes v. 3.2.4 (Huelsenbeck & Ronquist 2001) using jModelTest v. 2.1.7 (Darriba et al. 2012) to choose best-fit models of nucleotide substitution. The GTR+G model was chosen for both analyses.

A total of 1000 bootstrap replicates were performed to assess the relative robustness of the branches of maximum likelihood in RAxML. In Bayesian analysis, MCMC was performed for four chains and run for 1,0000,000 generations (sampling trees every 1000 generations) with a 25% burn-in.

Taxonomy

The alignment includes 96 ITS sequences representing 28 species of white-spored *Agaricaceae* and comprises 805 characters.

Both Maximum Likelihood and Bayesian analyses produced the same topology. Only the Bayesian tree with posterior probability and bootstrap values is shown (Fig, 1). *Leucoagaricus ariminensis* is a sister species of *Leucoagaricus ionidicolor* (MLB = 100; BPP = 1), with which it shares 91.7% of bases/residues. They form the sister clade of *Lepiota decorata* and are placed within /Piloselli. The sequence of *L. ionidicolor* from the Czech Republic specimen shares 658/663 (99%) nucleotides compared with the Dutch sequence (AY 176415).

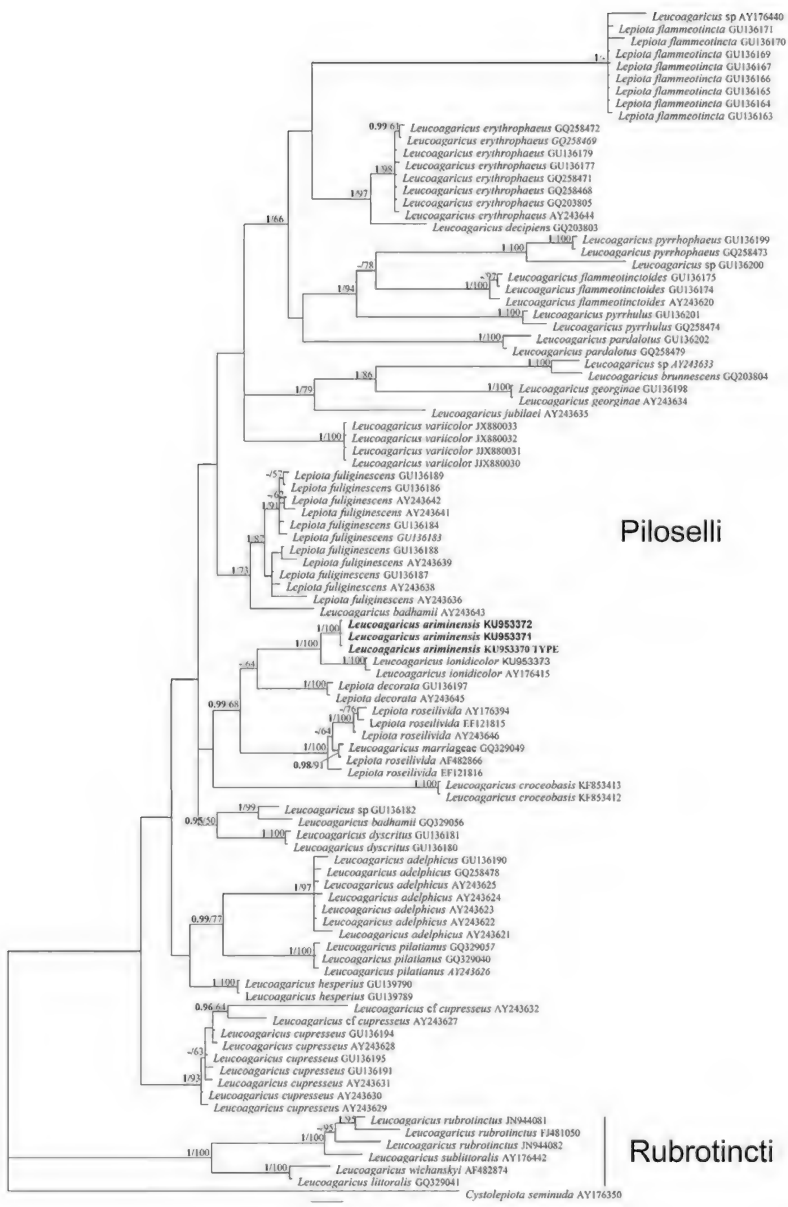

FIG. 1. Bayesian phylogram obtained from the general ITS sequence alignment of *Leucoagaricus/ Lepiota* spp.; *Cystolepiota seminuda* was used as outgroup taxon. Only BPP values >0.95 (in bold) and MLB values >50% are shown above clade branches. Sequences of *Leucoagaricus ariminensis* are in bold.

FIG. 2. *Leucoagaricus ariminensis* (holotype, MCVE 28443). Basidiomata. Scale bar = 20 mm.

Leucoagaricus ariminensis Dovana, Angeli, Contu & Brandi, **sp. nov.** FIGS 2–4

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Differs from *Leucoagaricus ionidicolor* by its more robust basidiomata, stipe that is usually shorter than the pileus diameter, more variably shaped and larger cheilocystidia, larger spores, and longer basidia.

TYPE: Italy, Emilia-Romagna, Rimini (RN), Parco XXV Aprile, along an avenue of cypress trees, 27.12.2014, legit L. Brandi (**Holotype**, MCVE 28443; GenBank KU953370).

ETYMOLOGY: the specific epithet "*ariminensis*" derives from Ărīmĭnum, Latin name for Rimini, a city in Emilia Romagna Region of the Italian Republic.

Pileus 3.5–8 cm, initially convex, becoming plano-convex, then depressed with age and plane in the centre; margin regularly involute at first, becoming incurved for a long time, then finally extended and somewhat irregularly sinuate; violet grey, violet brown-grey, violet (5P 3/4–8; 5P 4/4–8), tomentose, with tufts of thick hairs forming fine, densely packed squamules (lens), sparser at the margin and there the white flesh showing through. Negative reaction to aqueous ammonia.

Stipe 2–4(–4.5) × 1–2 cm, cylindrical, shorter than the pileus diameter, tapering upwards from an enlarged but not bulbous base, tapering downwards in some specimens, base well-covered with leaf-mould or debris, fibrous, fistulous; surface covered with white hairs faintly tinged with violet (5P 7/2-4)

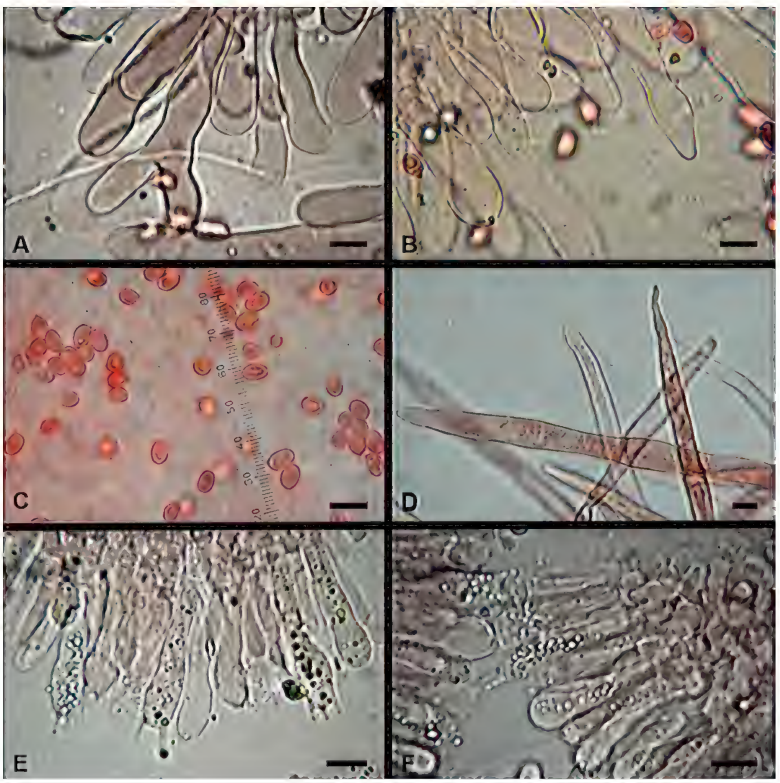

Fig. 3. *Leucoagaricus ariminensis* (holotype, MCVE 28443). A, B. Cheilocystidia. C. Spores. D. Terminal elements of the pileipellis. E. F. Basidia. Scale bars = 10 µm.

above the annulus, whitish and stained purple below (5RP 5/2–4)) where brown stains appear with age or handling. Annulus pendant, membranous, tomentose on the underside like the stipe surface, rim concolorous with the pileus.

Lamellae, L = 70–90, l = 2–3, moderately crowded, rounded and free at the stipe with a collarium, interspersed with numerous truncate lamellulae of various lengths; white to pale cream; edge sterile.

Flesh thick, tender, fragile, fibrous in the stipe, yellowing when handled or with age; odour faint, acidulous, only perceptible in young specimens, becoming unpleasant and smelling of fish with age; taste mild. Non-staining in contact with ammonia. Spore deposit light cream (2.5YR 8/1).

Basidiospores [270/3/4] (5.5–)6.5–8.1(–10.5) × (3.3–)4.1–4.9(–6.1) µm, on average 6.9–7.8 × 4.3–4.6 µm, Q = (1.18–)1.45–1.84(–2.31) Q_{av} = 1.5–1.8;

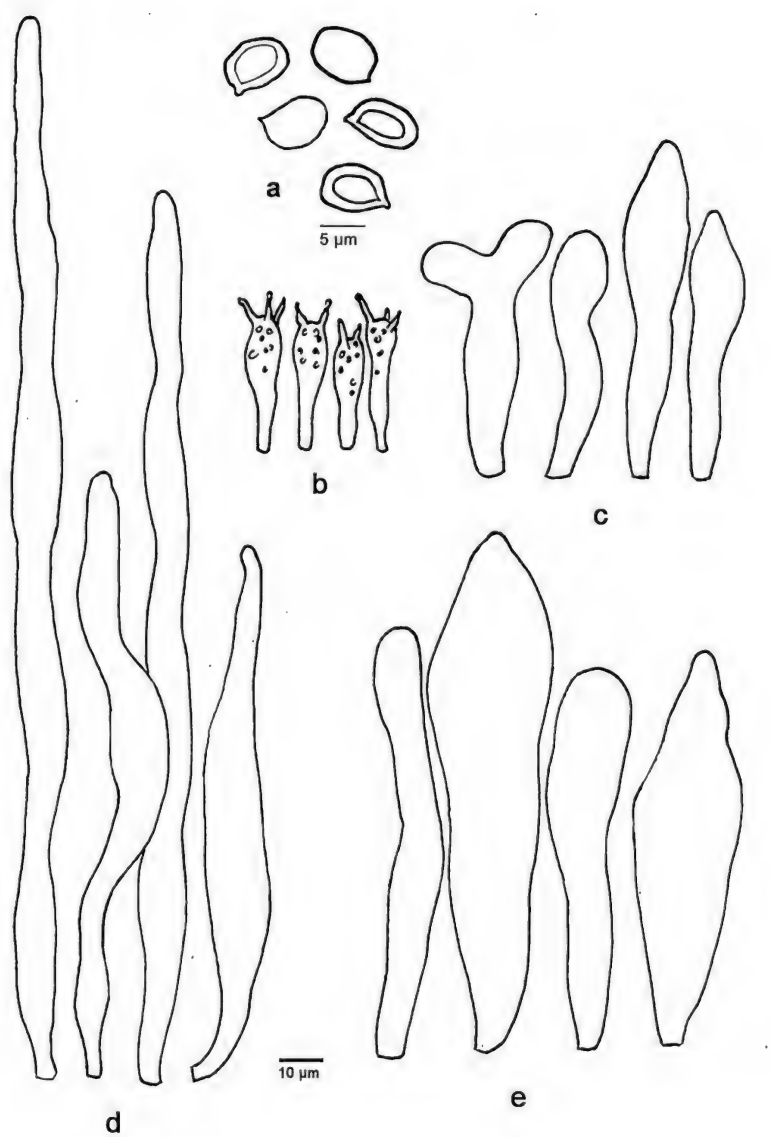

Fig. 4. *Leucoagaricus ariminensis.*
a Spores. b Basidia. c Cheilocystidia. d. Terminal elements of the pileipellis. e. Caulocystidia.
Scale bars: a = 5 µm; b–e = 10 µm. (Drawings by Maria Tullii).

mainly ellipsoid, with a rounded or sometimes subacute apex, some amygdaliform or subcylindrical, smooth, guttulate, thick-walled, without a germ pore or suprahilar depression, dextrinoid, metachromatic in Cresyl Blue, binucleate (rarely tetranucleate). Basidia 33.9–38.6 × 7.0–8.8 µm, 4-spored, some 2-spored, seldom 1-spored, at times somewhat swollen in the upper part. Lamellar trama ± regular, hyphae 5–10 µm wide, without clamps. Pleurocystidia not observed. Cheilocystidia 48–73 × 12–15 µm, abundant, variable in shape, from cylindrical, clavate with an obtuse apex to lageniform, rarely bifurcate. Pileipellis formed by repent interwoven hyphae, becoming a trichoderm near the squamules, with long, apically rounded terminal elements intermingled with elements that are tapered towards the apex, 150–330 × 7–12 µm; vacuolar pigment brownish-green-grey, at the centre some hyphae with granular vacuolar pigment; greenish-yellow excretory hyphae present. Non-gelatinised hyphae at disc. Subpellis formed by ± parallel hyphae, 9–16 (20) µm wide, slightly interwoven, with apically rounded, clavate and cylindrical terminal elements. Caulipellis formed by hyphae 5.9–13.8 µm wide, ± parallel, the outer ones interwoven with apically rounded, clavate and cylindrical terminal elements, some hyphae ending with long hairs; branched hyphae and some with excrescences also observed; caulocystidia lageniform, cylindrical, clavate, trunco-conical, 74–130 × 11–40 µm. Clamps absent.

Ecology: gregarious on bare sandy soil rich in vegetable litter (and animal excrements) under old cypress trees, *Cupressus sempervirens* L. In winter, from a single locality in Emilia Romagna.

Additional material studied: **ITALY**, **Emilia-Romagna**, **Rimini** (RN), Parco XXV Aprile: along an avenue of cypress trees, 12.12.2014, legit L. Brandi (MCVE29052; GenBank KU953372); 15.01.2015 legit A. Brandi (MCVE29053; GenBank KU953371).

Discussion

Leucoagaricus ariminensis has, as major morphological features, a rather fleshy predominately violaceous basidioma, usually with a stipe shorter than the pileus diameter and with a membranous purple-rimmed white annulus. It is distinguished by spores without a germ pore and a non-gelatinous pileus covering with somewhat elongated terminal elements that at times taper towards the apex. Since the basidioma surfaces in *L. ariminensis* react negatively to ammonia, this species would be placed in *Leucoagaricus* subsect. *Trichodermi* Bon & Migl. according to Bon's (1993) classification. However, our molecular results do not support this attribution.

ITS sequence analyses support *L. ariminensis* in *Leucoagaricus* sect. *Piloselli* (Kühner) Singer, indicating that a negative reaction to ammonia is not a constant within this section.

In both Bayesian (Fig. 1) and ML trees, *L. ariminensis* is distinct from *L. ionidicolor* Bellù & Lanzoni from the Netherlands (AY176415) and the Czech Republic (KU953373). Statistics support this separation (BPP = 1, MLB = 100%), and sequences from both *L. ionidicolor* collections differ from *L. ariminensis* by at least 42 nucleotides.

The ITS sequence from the Czech *L. ionidicolor* basidioma differs from that of the Dutch basidioma by only 5 nucleotides. The Czech specimen has "terminal cells which are narrowly clavate when young, then very long, 80–200 × 15–20 µm narrowly fusiform with rostrate apex" (Holec 2009), unlike the pileipellis of *L. ariminensis* where no rostrate elements are present.

Compared to *L. ariminensis, L. ionidicolor* has a more slender stem, a minutely squamulose pileus surface, and shorter basidia, (14–21 × 7–8 µm, Bellù & Lanzoni 1988; 14–21 × 5.5–6.5 µm, Vellinga 2001), smaller, ellipsoid to oblong basidiospores ((5–)5.5–6.5 × (3–)3.3–3.7(–4) µm, Bellù & Lanzoni 1988; 6.0–7.0 × (3.5–)4.0–4.5 µm, Vellinga 2001), and smaller clavate cheilocystidia (23–45 × 8–11(–12) µm, Bellù & Lanzoni 1988; 23–40 × 8.0–10 µm often with curved pedicel, Vellinga 2001). By contrast, the *L. ariminensis* spores range from ellipsoid to oblong with a rounded apex, are rarely amygdaliform, and show a great variability with regard to their dimension and Q within different basidiomata.

Leucoagaricus ionidicolor var. *major* J. Charb. et al., which differs mainly from the type species by its larger pileus and deeper shade of violet / purple, is very similar to *L. ariminensis* from which it can be distinguished by its slightly smaller spores (6–7 × 4–4.5(5) µm), shorter (100(–150) µm) fusiform terminal pileipellis elements, and smaller and more uniformly shaped cheilocystidia (Bon & Charbonnel 2000; no basidium dimensions provided). Further molecular studies are needed to clarify the position of this variety within the *L. ionidicolor* group.

Bon (1993) placed two more violaceous-coloured species in *Leucoagaricus* subsect. *Trichodermi: L. ianthinophaeus* Locq. and *L. ianthinosquamulosus* Guinb., both described from France.

Leucoagaricus ianthinophaeus, still not well-known possibly because of its rarity, can be distinguished from *L. ariminensis* by its slender white glabrous stipe with no trace of an annulus, smaller spores, inconspicuous cheilocystidia, and its pileus covering composed of much shorter hyphae with terminal

elements 30–50 × 5–10 µm according to Bon (1993), who re-examined Locquin's original material. *Leucoagaricus ianthinosquamulosus* can easily be distinguished by its smaller basidiomata, definitely larger subfusiform spores (6.5–10(–12.5) × 4–5(–6) µm), shorter (16–50 µm) and more variably shaped cheilocystidia, and encrusting pigments in the pileus covering (Bon 1993, Guinberteau 1993).

Leucoagaricus idae-fragum Guinb. et al., a pink European species synonymized with *Lepiota decorata* based solely on morphology by Vellinga (2006), differs mainly in its remarkable raspberry red cap colours, the presence of a persistent general veil, a green ammonia reaction on gills and cap, and the differently shaped cheilocystidia (Guinberteau et al. 1998).

Lepiota roseilivida Murrill from western North America (= *Leucoagaricus marriageae* (D.A. Reid) Bon from Europe) can be diagnosed immediately by its slender appearance, spores 6.7–9.8 × 3.8–5.7 µm that are oblong or amygdaliform in profile, and green-staining reaction of the external basidioma surfaces to ammonia (Vellinga 2006).

Leucoagaricus brunneolilacinus Babos is a brownish lilac species with a negative ammonium reaction. It becomes distinctly orange when bruised and differs mainly from *L. ariminensis* by smaller spores (4–)4.2–5.1(–5.4) × 2.3–3.2(–3.5) µm, shorter (13–26 µm) cylindrical to clavate cheilocystidia, and a pileipellis with shorter (6–60 × 5–12(–16) µm) clavate terminal elements that in young basidiocarps are intermixed with scattered globose cells (Candusso & Lanzoni 1990).

Two other *Leucoagaricus* species with lilac basidiomes, *L. purpureolilacinus* Huijsman from Europe and *L. subpurpureolilacinus* Z.W. Ge & Zhu L. Yang from Asia, are phylogenetically well separated from /Piloselli and differ from *L. ariminensis* mainly by their clavate cheilocystidia crowned with crystals and different pileipellis (Vellinga 2001, Ge et al. 2015).

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Geastrum reinkingii reconsidered

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Abstract—Morphological studies based on type specimens revealed that *G. reinkingii* (originally described from Honduras, but subsequently synonymized under *G. lloydianum*) is a good species that is probably well distributed in tropical and subtropical forests in America. Here we present a modern description and color photographs of the species, and a comparison with the morphologically closest South American species: *G. glaucescens*, *G. hariotii*, *G. lloydianum*, and *G. setiferum*.

Key words—*Agaricomycetes,* earthstar fungi, gasteromycetes, *Geastraceae*, Neotropical mycota

Introduction

Geastrum Pers. is a widespread genus with numerous taxonomical problems. Many authentic species have been treated as synonyms (Trierveiler-Pereira & Silveira 2012, Zamora et al. 2014); and sometimes different species have been identified under a single name (e.g., in *G. fimbriatum* Fr.) due to a lack of differentiating morphological characters (Kasuya et al. 2012).

Several *Geastrum* species collected in tropical and subtropical areas of America were described by C.G. Lloyd, who during many years received specimens from contributors. The type specimen of *G. reinkingii* was collected in 1923 in Honduras (Central America). Its original publication (Lloyd 1924) does not include a full description of the specimen, but instead, briefly differentiates it from *G. hariotii* Lloyd. Later, the name also appeared, with no additional information, in Rick's posthumous list of the gasteroid species found

in southern Brazil (Rick 1961). Finally, the species was considered a synonym of *G. lloydianum* Rick by Ponce de León (1968) in his revision of the family *Geastraceae*.

Recent morphological studies based on *Geastrum* type specimens revealed that *G. reinkingii* is distinct from *G. lloydianum* and should be considered an authentic species.

Materials & methods

In order to better circumscribe the species, recently collected specimens and vouchers from herbaria BPI, ICN, LPS, PACA (Fungi Rickiani), and URM (Thiers 2016) were examined macro- and microscopically. Macroscopic characters were described based on observations of fresh and dried material, according to Sunhede (1989). Colors and measurements refer to dry material unless otherwise stated. Colors of macroscopic structures were determined according to Kornerup & Wanscher (1978). Colors of microscopic features were defined in KOH preparation.

Observations of microscopic characters were made under a light microscope on glass slide mounts (in 5% KOH) prepared from dried specimens. Scanning electronic microscopy (SEM) was conducted at 'Centro de Microscopia Eletrônica', UFRGS. Basidiospores were coated with 15 nm of gold using a Bal-Tec SCD050 sputter coater and photographed with a JEOL JSM-5800 scanning electron microscope.

Taxonomy

Geastrum reinkingii Lloyd, Mycol. Writings 7: 1280. 1924 [as "*Geaster*"]. Pl. 1

Basidiomata 2.0–3.4 cm high when expanded. Exoperidium non-hygroscopic, 1.4–2.7 cm high × 2.8–4.2 cm broad, split into 6–8 rays, some ramifying at the tip, arched; mycelial layer pale yellow (1A3), usually peeling off at maturity, if persistent, encrusted with debris; fleshy layer usually not persistent, pale orange (5A3) to brownish orange (5C3) when fresh, then yellowish brown (5D5) to brown (6E5) when dry; fibrous layer brownish orange (5C5) to light brown (5D4). Endoperidium subglobose to globose when recently exposed, then depressed globose to ellipsoid, 0.5–1.2 cm high (not including the peristome) × 1.4–2.0 cm broad, yellowish brown (5E4) to grayish brown (5E3, 6E3), when fresh, then yellowish brown (5D4) to dark brown (6F3) when dry, stalked; stalk short, up to 1.0 mm high, cylindrical or flattened, dark blond (5D4) to yellowish brown (5D5), endoperidial surface asperulate to rough due to the presence of conical fascicules of hyphae (most easily observed at the base of the endoperidium, near the apophysis); apophysis beige (4B3) to yellowish brown (5D5); peristome deeply sulcate, usually strongly delimited, up to 0.45 cm high, concolor or slightly darker than endoperidium, almost black in some specimens. Gleba pulverulent at maturity, dark brown (6F4).

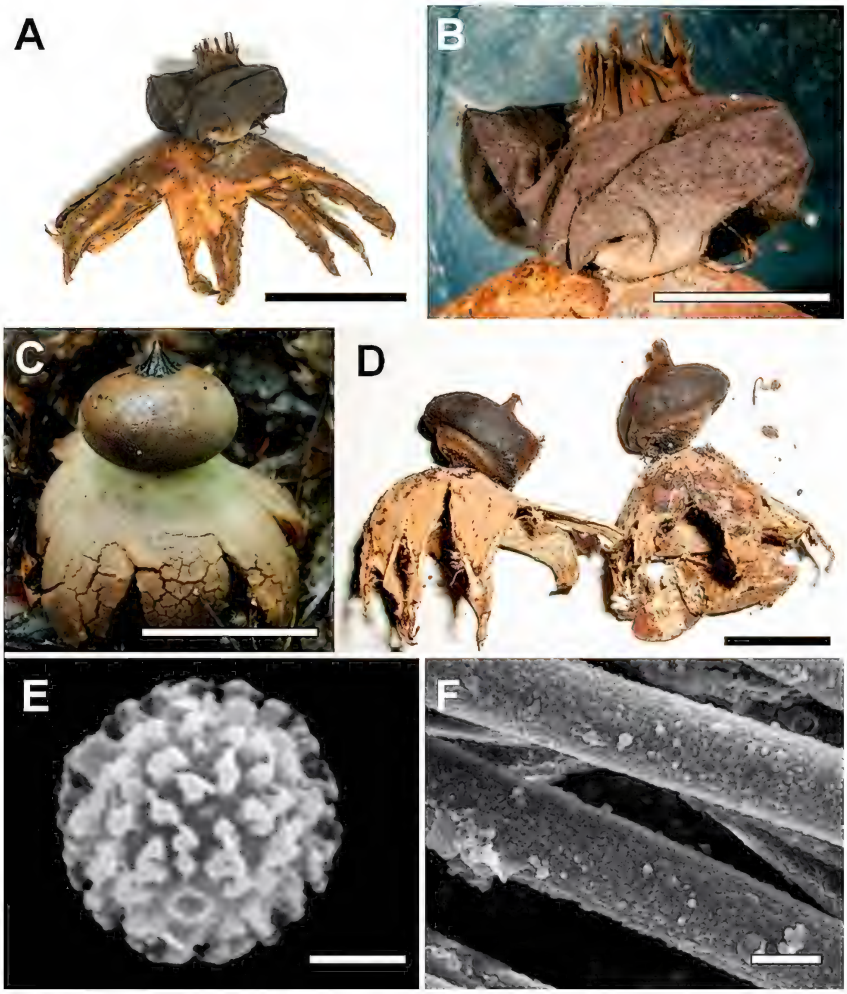

Plate 1. *Geastrum reinkingii*. A. Holotype (BPI 705664). B. Detail of the endoperidium (holotype). C. Fresh basidiome (ICN 177057). D. Dried specimens (URM 82017). E. Basidiospore (from the holotype). F. Capillitial hyphae (from the holotype). Scale bars: A, C, D = 2 cm; B = 1 cm; E = 1 µm; F = 2.5 µm.

Capillitial hyphae ≤8 µm wide, pale yellow, brown to golden brown, straight, not branched, thick-walled with narrow lumen, tips round, with or without encrusted material. Basidiospores globose, 3.5–4.5 µm in diam. (including ornamentation), brown to dark brown, ornamented with short

columns, some confluent; basidia not seen. HYPHAE OF THE MYCELIAL LAYER ≤3 µm wide, hyaline to pale yellowish, thick-walled; hyphae of the fibrous layer 3–11 µm wide, yellowish to pale brown, thick-walled to solid; HYPHAE OF THE PSEUDOPARENCHYMATOUS LAYER globose to ellipsoid, ≤74 µm in the largest dimension, hyaline to yellowish; HYPHAE OF THE ENDOPERIDIUM ≤10 µm wide, yellowish to pale brown, thick-walled to solid, the ones forming the hyphal fascicules of the surface are tortuous, tightly interwoven with other hyphal elements (mycosclerids).

HABITAT—growing solitary or in small groups (four or five basidiomata) on forest soil (litter), without forming a subiculum.

DISTRIBUTION—known from Honduras and tropical and subtropical areas of Brazil. It is very likely that the species occurs throughout the Neotropical rainforests and warmer regions of temperate America.

SPECIMENS EXAMINED—**HONDURAS. ATLANTIDA, Tela,** 20.III.1923, leg. O.A. Reinking (BPI 705664, holotype!). **BRAZIL. PERNAMBUCO, São Vicente Férrer,** Mata do Estado, 18.IX.2008, leg. L. Trierveiler-Pereira & Baltazar LTP180 (URM 82017, as *G. lloydianum*; ICN 177060); **RIO GRANDE DO SUL, São Leopoldo,** leg. J. Rick (BPI 705665); 1933, leg. J. Rick (PACA 15976, as *G. berkeleyi*); **Caçapava do Sul,** Parque Municipal Pedra do Segredo, 6.VI.2011, leg. L. Trierveiler-Pereira LTP277 (ICN 175626); **PARANÁ, Foz do Iguaçu,** Parque Nacional do Iguaçu, Trilha das Bananeiras, 9.II.2011, leg. L. Trierveiler-Pereira LTP179 (ICN 177057).

ADDITIONAL SPECIMENS EXAMINED—***Geastrum glaucescens***: **ARGENTINA. BUENOS AIRES, La Plata,** IX.1894, leg. C. Spegazzini (LPS 15860, holotype).

Geastrum hariotii: **BRAZIL**. C.G. Lloyd's Collection cat. No. 52535 (BPI 841469, holotype).

Geastrum lloydianum: **BRAZIL**. Leg. J. Rick, C.G. Lloyd's Collection cat. No. 57279 (BPI 841471, holotype).

Geastrum setiferum: **BRAZIL. PERNAMBUCO, Serra Negra,** 28.V.2002, leg. I.G. Baseia (URM 77077, paratype); **PARAÍBA, Mataraca,** Mineradora Millenium (Cristal), mata controle, 2.IX.2009, leg. L. Trierveiler-Pereira (URM 82118).

COMMENTS—*Geastrum reinkingii* is characterized by medium-sized basidioma, arched exoperidium; usually not persistent mycelial and fleshy layer; subglobose to ellipsoid, stalked endoperidium, with dark, asperulate to rough endoperidial surface, and a whitish apophysis.

The endoperidial stalk and the apophysis are not clearly evident when the endoperidium is recently exposed, but both are remarkable features in dried collections. Some herbarium specimens exhibit a whitish pruina on the endoperidial surface. The basidiospores are typically dark brown and with discrete ornamentation. Immature forms were not found in herbarium collections or in the field, therefore basidia morphology remains unknown. Brazilian specimens show no major differences from the holotype, the whitish

apophysis and short-stalked blackish depressed globose endoperidium being the most remarkable features. Basidiospore measurements and morphology are constant among the examined specimens.

One *G. reinkingii* collection from Southern Brazil, sent by Rick to Lloyd, is at BPI (705665), but no collections were deposited at PACA, Rick's most important fungal collection. However, one of Rick's collections identified as *G. berkeleyi* (PACA 15976) does correspond to *G. reinkingii*. One *G. reinkingii* specimen (URM 82017) was reported from Northeastern Brazil as *G. lloydianum* (Trierveiler-Pereira et al. 2011).

Geastrum hariotii and *G. lloydianum* are very closely related species, and their differences have been discussed by Trierveiler-Pereira & Silveira (2012). Microscopically, the differences separating *G. reinkingii* and *G. lloydianum* are evident, since the *G. lloydianum* basidiospores are 5–6 µm in diam., yellowish brown, and prominently ornamented. Since there are a limited number of morphological characters available to differentiate species of *Geastrum*, we still believe that basidiospore morphology is an important diagnostic feature. The *G. hariotii* basidiospores are more similar to those of *G. reinkingii*; nonetheless, the two species are macroscopically distinct: *G. hariotii* produces smaller basidiomata with a globose sessile, not asperulate, endoperidium. Moreover, no whitish apophysis is observed in *G. hariotii*.

Geastrum setiferum Baseia produces large arched basidiomata and a short stalked endoperidium (Baseia & Milanez 2002, Trierveiler-Pereira et al. 2011). In many aspects it resembles *G. reinkingii*, but the presence of dark setae in the *G. setiferum* endoperidium is an important distinguishing feature. Moreover, the endoperidial surface of *G. setiferum* is lighter (usually light brown), not remarkably ellipsoidal, without a whitish apophysis, and the peristome morphology is a transition between fibrillose and plicate.

Geastrum glaucescens Speg. was described from Argentina and its basidiomes are small, arched, and dark colored. The endoperidium is globose, brownish to blackish, and covered with abundant persistent white mesoperidial matter (Kuhar et al. 2013). According to the protologue, the endoperidium is short stalked (Spegazzini 1912), but this feature was difficult to confirm by analysis of the type specimen, which is sectioned in half. Soto & Wright (2000) illustrated the species as clearly stalked. The primary macroscopical features distinguish this species from *G. reinkingii*, as depicted by Kuhar et al. (2012).

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Bacidia, Micarea, Sagedia, and *Stigmidium* species new to Turkey

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Abstract—During lichenological exploration in Turkey, two lichenised fungi (*Micarea micrococca, Sagedia zonata*) and one lichenicolous fungus (*Stigmidium microspilum*) were established as new records for Asia, and one lichenised fungus (*Bacidia chloroticula*) as new for Turkey. Illustrations are accompanied by brief descriptions and comments on habitat, substrate, and geographical distribution.

Key words—biodiversity, *Corylus*, Giresun, İstanbul, Trabzon

Introduction

Studies on the lichen biodiversity of Turkey are as yet incomplete, but many new lichenized and lichenicolous fungi records for Turkey have been found in recent years (Kınalıoğlu & Aptroot 2010, Kocakaya et al. 2011, Karagöz & Aslan 2012, Aslan & Yazıcı 2013, Halıcı 2015, Yazıcı & Aptroot 2015, Şenkardeşler et al. 2016). Despite the increase in the number of lichen records, additional studies are needed in some places where data on lichen biodiversity are still scarce. The objective of this paper is to add new species to the list of lichens known from Turkey.

Materials & methods

The study is based on lichen material collected in three different Turkish provinces (Giresun, İstanbul, Trabzon). Vouchers are preserved in the herbarium of the Biology Department, Faculty of Science and Arts, Giresun University, Giresun, Turkey (GUB).

Morphological, anatomical, and chemical features of the lichen thalli were examined with a stereomicroscope, a light microscope and the standard chemical spot tests (e.g. 10% potassium hydroxide).

Species recorded

Bacidia chloroticula (Nyl.) A.L. Sm., Monogr. Brit. Lich. 2: 155. 1911. FIG. 1

Thallus thin, grey–pale green. Photobiont cells 5–12.5 µm diam. Apothecia small, flat, 0.07–0.3 mm diam., pinkish to pale brown. Hymenium colourless, 32.5–40 µm tall, epihymenium and hypothecium colourless. Ascospores 1–4-celled, 20–35 × 1–1.6 µm, acicular. Thallus C–, K–, KC–, PD–.

SPECIMEN EXAMINED: **TURKEY.** İStanbul, **Maltepe,** in the vicinity of Başıbüyük, 40°57′32″N 29°09′27″E, 235 m, on siliceous rocks, 24 April 2007, Kınalıoğlu (GUB 2383).

REMARKS—For a detailed description of the species see Smith et al. (2009).

Bacidia *chloroticula* is a pioneer species. It grows on various shaded, often man-made substrata, such as tree bases and exposed roots, dead grasses, tops of cut stumps and fence posts, cement and brick rubble, and even painted

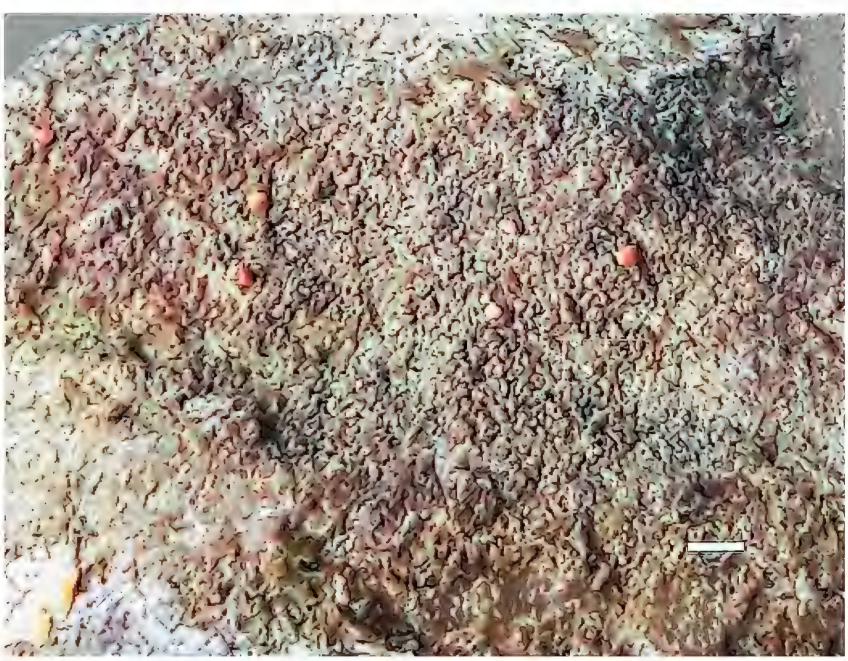

FIG. 1. *Bacidia chloroticula* (GUB 2383): habit. Scale bar = 1 mm.

iron-work and tarmacadam (Smith et al. 2009). In Turkey, we have recorded it only once, from siliceous rocks at low elevation.

Known previously from Asia, Europe, and North America (Smith et al. 2009). New to Turkey.

Bacidia chloroticula is most easily confused with Bacidina saxenii (Erichsen) M. Hauck & V. Wirth, which has a very similar ecology. It differs in having often darker, pale to dark-brown apothecia with pale brown hymenium and larger, vesicle-like, 20 × 12 mm large cells in the outer exciple (Smith et al. 2009). In *B. chloroticula* the cells in the exciple do not exceed 5(–7) µm in width (Smith et al. 2009). The size of the apothecia and hymenium in the Turkish collection is almost the same as given by Smith et al. (2009; apothecia 0.08–0.2 mm. diam., hymenium 40–42 µm). However, the ascospores in the Turkish material are slightly smaller than those reported by Smith et al. (2009; 30–50 × 2–3µm).

Micarea micrococca (Körb.) Gams ex Coppins,
Checkl. Lich. Gr. Brit. Ire.: 86. 2002. FIG. 2

Thallus crustose, greenish. Apothecia convex to subglobose, immarginate, mostly tuberculate, 0.1–0.3 mm diam., to 0.5 mm diam. when tuberculate, pinkish to flesh or rarely pale brownish. Epihymenium colourless, K–, hymenium 32.5–45 µm tall, colourless. Asci clavate, 8-spored. Ascospores ellipsoid to oblong, hyaline, 1-celled, 7.5–10 × 2.5–3 µm. Thallus and apothecia C–, K–, KC–, PD–.

SPECIMEN EXAMINED: **TURKEY. GİRESUN: Espiye,** Çepniköy, 40°51′38″N 38°43′57″E, 493 m, on garden fence, 5 October 2013, Kınalıoğlu (GUB 2384).

REMARKS—For detailed descriptions of the species see Smith et al. (2009) and Barton & Lendemer (2014).

Micarea micrococca, the most common representative of the *M. prasina* group, is found on acid bark of trees and stumps, shrubs, plant debris (usually in conifer plantations), soil or debris in rock crevices in coastal sites, rarely on shaded sandstone rocks, mostly in shaded sites or niches, even close to large city centres (Smith et al. 2009). In Turkey, *Micarea micrococca* was collected on a garden fence next to a shady hazelnut field.

Known previously from Australia, Europe, and North America (Smith et al. 2009). New to Asia.

Micarea micrococca is extremely variable. It is very similar to *M. prasina* Fr. and can sometimes only be separated by using TLC. The apothecia are the clearest morphological character differentiating *M. micrococca* from

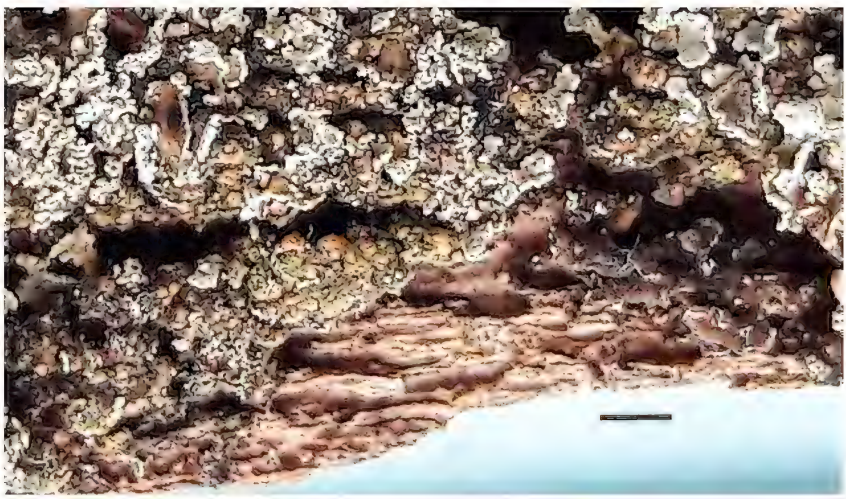

Fig. 2. *Micarea micrococca* (GUB 2384): habit. Scale bar = 1 mm.

M. prasina, which has whitish, smaller apothecia (≤0.2 mm diam.; Smith et al. 2009, Dobson 2011).

The ascospores and hymenium in the Turkish specimen are smaller than those cited by Barton & Lendemer (2014; ascospores 7.4–10.9 × 2.3–3.6 µm, hymenium 40–60 µm) and Smith et al. (2009; ascospores 6–17 × 2–4.5 µm, hymenium 30–50 µm). The Turkish collection differs from North American samples by the K– reaction.

Sagedia zonata Ach., Kongl. Vetensk. Acad. Nya Handl. 30: 165. 1809. Fig. 3

Thallus crustose, saxicolous, greyish or very slightly bluish, irregularly cracked-areolate, areoles to 1.5 mm wide. Apothecia black, 0.25–1 mm diam., numerous, immersed or somewhat raised, with a prominent black rim. Hymenium 80–95 µm tall, colourless. Asci 8-spored. Ascospores simple, mostly subglobose, broadly ellipsoid or very rarely ellipsoid, 13.5–20 × 7.5–11 µm. Thallus C–, K–, KC–, PD–.

Specimen examined: **TURKEY**. Trabzon: **Araklı**, S of Kızılkaya Yaylası, 40°39′56″N 40°01′49″E, 2300 m, on siliceous rocks, 17 August 2005, Kınalıoğlu (GUB 2385).

Remarks—For a detailed description of the species see Wirth et al. (2013).

This boreal-temperate species grows on non-calcareous blocks, rocks, and boulders in slightly shady to mostly well-lit areas, but also on periodically or

Fig. 3. *Sagedia zonata* (GUB 2385): habit. Scale bar = 1 mm.

rarely inundated surfaces next to streams in montane to subalpine zones (Wirth et al. 2013). In Turkey, we collected it from siliceous rocks in an exposed area.

Known previously from Europe (Wirth et al. 2013). New to Asia.

The Turkish material of *Sagedia zonata* differs from European specimens by having bigger areoles and a lower hymenium. In the European collections the areole is 0.5–1 mm diam., and the hymenium is 85–100 µm high (Wirth et al. 2013). Otherwise the Turkish collection matches the sizes given by Wirth et al. (2013; apothecia 0.2–1 mm diam., ascospores 14–19 × 8–12 µm).

Stigmidium microspilum (Körb.) D. Hawksw., Kew Bull. 30(1): 201.1975. Fig. 4

Thallus dark brownish circular patches, patches ≤0.5–1.5 mm wide, regularly scattered on the thallus surface of the host (*Graphis scripta* (L.) Ach.). Perithecia small, black, 0.1–0.12 mm diam. Ascospores hyaline, elongate or narrowly ellipsoid, 2-celled, 14–18.8 × 2.5–3.8 µm. Thallus C–, K–, KC–, PD–.

Fig. 4. *Stigmidium microspilum* (GUB 2386): habit. Scale bar = 1 mm.

Specimen examined: **TURKEY. Giresun: Espiye,** N of Yeniköy village, 40°4′42″N 38°45′31″E, 781 m, on the thallus of *Graphis scripta* on *Corylus* sp., 6 October 2013, Kınalıoğlu (GUB 2386).

Remarks—For a detailed description of *S. microspilum*, see Hawksworth (1983).

This widespread lichenicolous species occurs on thalli of *Graphis scripta* on *Corylus* sp. in old woodlands (Hawksworth 1983). In Turkey it was growing on a *Graphis scripta* thallus in a damp hazelnut garden.

Known previously from Europe (Hawksworth 1983). New to Asia.

The Turkish material of *Stigmidium microspilum* has slightly longer and narrower ascospores than those described from European material (Hawksworth 1983; ascospores 14–15 × 3–5 μm).

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Urocystis narcissi, a new record from Asia

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Abstract—*Urocystis narcissi,* reported here from Pakistan, represents a new record for Asia. This smut fungus was collected for the first time outside its type locality in Spain. *Narcissus tazetta* is recognized as a new host plant for this rare smut.

Key words—Shangla, Swat, taxonomy, *Ustilaginales*, *Ustilaginomycetes*

Introduction

During a survey of *Ustilaginales* in the Shangla and Swat districts of Khyber Pakhtunkhwa (KP) Province, Pakistan, *Narcissus tazetta* was found infected with *Urocystis narcissi*. This rare smut fungus was previously known only from its type locality in Spain. The sampling sites are neighborhood districts located at 34°34′–35°55′N 71°08′–72°50′E. *Narcissus tazetta* was found infected with smut in two areas of Khyber-Pakhtunkhwa (Ahmad & Sirajuddin 1996): one infected sample was collected from Kabal valley, Swat, in February 2015, and the second from Puran valley, Shangla, in March 2015. Nine smut fungi have previously been reported from Swat, but none from Shangla (Ahmad et al. 1997).

Materials & methods

After collection of the infected plants with their inflorescences, the specimens were photographed, and preserved. Free hand sections were cut and scrape mounts of the infected portions were observed under a Labomed CSM2 stereomicroscope. For light microscopic (LM) observations, spores were mounted in lacto phenol solution on glass slides, gently heated to boiling point to rehydrate the spores, and then cooled.

Preparations were examined using a Nikon YS 100 microscope, and photographed with the aid of a Digipro-labomed. Forty spores were measured using a Zeiss eyepiece screw micrometer. For SEM, the spores were critically point dried, attached to specimen holders with double sided adhesive tape, and coated with a 50 nm gold film in a Polaron E5300 freeze drier. The gold-coated specimens were observed and photographed in a Camscan 3-30BM scanning electron microscope. Specimens have been deposited in the herbaria at the University of the Punjab, Lahore, Pakistan (LAH) and Hazara University Mansehra, Pakistan (HUP).

Fig.1: *Urocystis narcissi.* A, B, D. Infected host plants. C, E. Spore balls in LM. Scale bars: A, B = 51 µm; C, D=10 µm; E=11 µm; F= 8 µm.

Taxonomy

Urocystis narcissi (Gonz. Frag.) Vánky, Mycotaxon 48: 41 (1993) FIGS 1, 2

≡ *Urocystis colchici* f. *narcissi* Gonz. Frag., Trab. Mus. Nac. Cienc. Nat., Ser. Bot. 7: 22 (1914)

≡ *Turburcinia narcissi* (Gonz. Frag.) Cif., Fl. Ital. Crypt., Fungi 1(17): 101 (1938)

Sori in leaves and scales, yellowish-silver or yellowish-brown or black pustules containing a black powdery mass. Spore balls globose to subglobose or ellipsoid, 25–44 × 31–54 µm, composed of 1–3(–4) spores, completely surrounded by sterile cells. Spores globose to subglobose, ovate or ellipsoidal, 12–21 × 13–24 µm, dark brown, walls smooth; sterile cells variable in shape and size, subglobose to elongated, 4–14 µm long, wall 0.6–1.5 µm thick, yellowish brown to hyaline, smooth.

SPECIMENS EXAMINED: On *Narcissus tazetta* L. (*Amaryllidaceae*): **PAKISTAN**, **KHYBER PAKHTUNKHWA PROVINCE**, **Swat District**, Kabal valley Deolai, 1000 m a.s.l , February 2015, leg. Sadiqullah SUS-02 (HUP 315); **Shangla District**, Puran Aloch, 1100 m a.s.l., March 2015, leg. Sadiqullah SUS-03 (HUP 316).

COMMENTS—*Urocystis narcissi* has previously been reported on *Narcissus pallidulus* Graells from the Sierra de Guadarrama, Spain (González Fragoso 1914, as *U. colchici* f. *narcissi;* Ciferri 1938, as *Turburcinia narcissi;* Vánky 1993, 2011). Our collections on *N. tazetta* from Pakistan are similar to those cited in

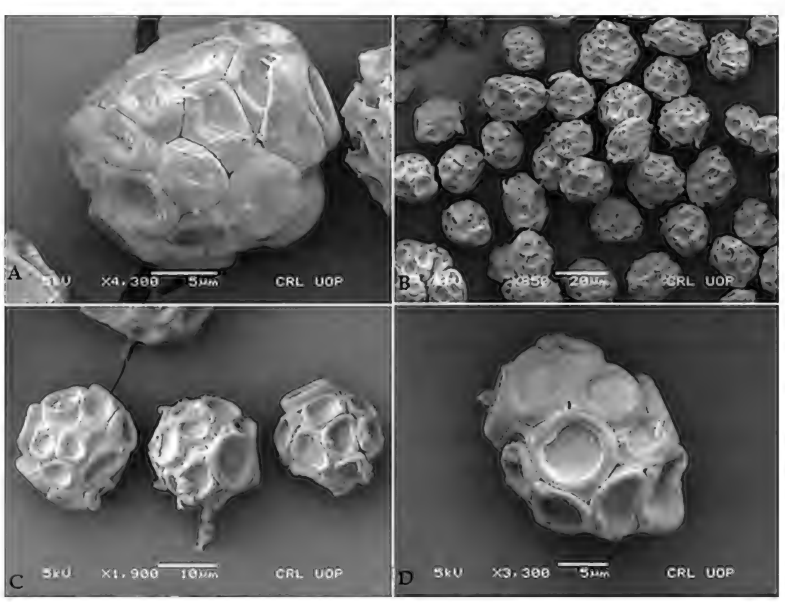

FIG. 2: *Urocystis narcissi.* Spore balls in SEM.

Vanky (2011), except that the maximum spore size in our material was slightly larger. *Narcissus tazetta* is a new host record for this fungus.

Acknowledgments

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Lembosia dianesei sp. nov. associated with *Peritassa campestris* in Minas Gerais, Brazil

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Abstract—A new *Lembosia* species is described in association with *Peritassa campestris* from a cerrado region of Minas Gerais, Brazil. The new species is described based on morphological characteristics and compared with other *Lembosia* species associated with celastraceous hosts.

Key words— *Ascomycota*, *Asterinaceae*, *Celastraceae*, cerrado

Introduction

The plant genus *Peritassa* Miers (*Celastraceae* R. Br.) includes nine species endemic to Brazil. These species are distributed across many Brazilian states and are found in the cerrado, cerradão, and campo ralo biomes. *Peritassa campestris* has been used pharmacologically, and the local population consumes the fruit in natura. However, since 2009 the leaves of some *P. campestris* plants in the Cristália and Botumirim municipalities of Minas Gerais have been found to have a black colored fungal colonization. This infection occurs most frequently during the rainy season. The fungal colonies have macroscopic characteristics similar to those of *Meliolales* Gäum. ex D. Hawksw. & O.E. Erikss. However, laboratory observations revealed that the fungus represents a species of *Lembosia* Lév., (*Asterinaceae* Hansf.). This genus is marked by the presence

of hyphopodiated mycelium, linear hysteriothecia with radiate upper walls, parallel bitunicate asci, and light brown bicellular ascospores (Bezerra 2004, Hofmann & Piepenbring 2014, Song & Hosagoudar 2003). Additionally, the identity of the host plant is required to distinguish *Lembosia* species (Inácio & Cannon 2003, Müller & von Arx 1962, Mebey & Hawksworth 1995). *Asterinaceae* are usually associated with tropical and subtropical plants around the globe (Hofmann & Piepenbring 2014, Guatimosim et al. 2015). Infection has been reported in wild plants but also occurs in cultivated plants, such as *Asterina manihotis* Syd. on living leaves on *Manihot esculenta* Crantz (Hofmann & Piepenbring 2008). New species of *Asterinaceae* have been described from Brazil, including *Lembosia bezerrae* Firmino & O.L. Pereira and *L. epidendri* Meir. Silva & O.L. Pereira (Silva & Pereira 2008, Firmino & Pereira 2014). Here we propose another new species, *Lembosia dianesei*, described and illustrated here.

Materials & methods

Peritassa campestris leaves were collected and sent to the Electron Microscopy and Ultrastructural Analysis Laboratory at the Universidade Federal de Lavras (LME/UFLA). Fragments and manual sections were mounted on slides in lactoglycerol for light microscopy examination of fungal structures. Melzer´s reagent was used to observe the amyloid zones of the asci. Permanent slides were made using PVLG (Polyvinyl alcohol-lactic acid-glycerol). Observations were made with a Leica DM2000 bright field vertical microscope using LAS v.4.5 software.

The leaf fragments used for scanning electron microscopy studies were fixed in Karnovsky solution (pH 7.2), post-fixed with 1.0% osmium tetroxide solution, and dehydrated in an acetone series (Bozzola & Russell 1999). The specimens were dried in a Bal-tec CPD 030 critical point dryer and coated with gold in a Bal-tec SCD 050 sputter coater for observation using the LEO EVO 40 and Smart Sem softwareat20 Kv scanning electron microscope with a working distance of 9 mm. Both light and scanning electron micrographs were edited in Corel Draw X7.

Two representative specimens were deposited in the herbarium of the Universidade Federal de Viçosa, Minas Gerais, Brazil (VIC).

Taxonomy

Lembosia dianesei M.P. Melo, J.L. Bezerra, S.I. Moreira & E. Alves, **sp. nov.** Pl. 1

MycoBank MB 814528

Differs from *Lembosia albersii* by its smaller asci and its supra-median ascospore septum; and from *L. lophopetali* and *L. salaciae* by its smaller asci and its shorter unicellular hyphopodia.

Type: Brazil, Minas Gerais, Cristália, on living leaves of *Peritassa campestris* (Cambess.) A.C. Sm. (*Celastraceae*), 30 July 2015, M.P. Melo (**Holotype**, VIC 42934).

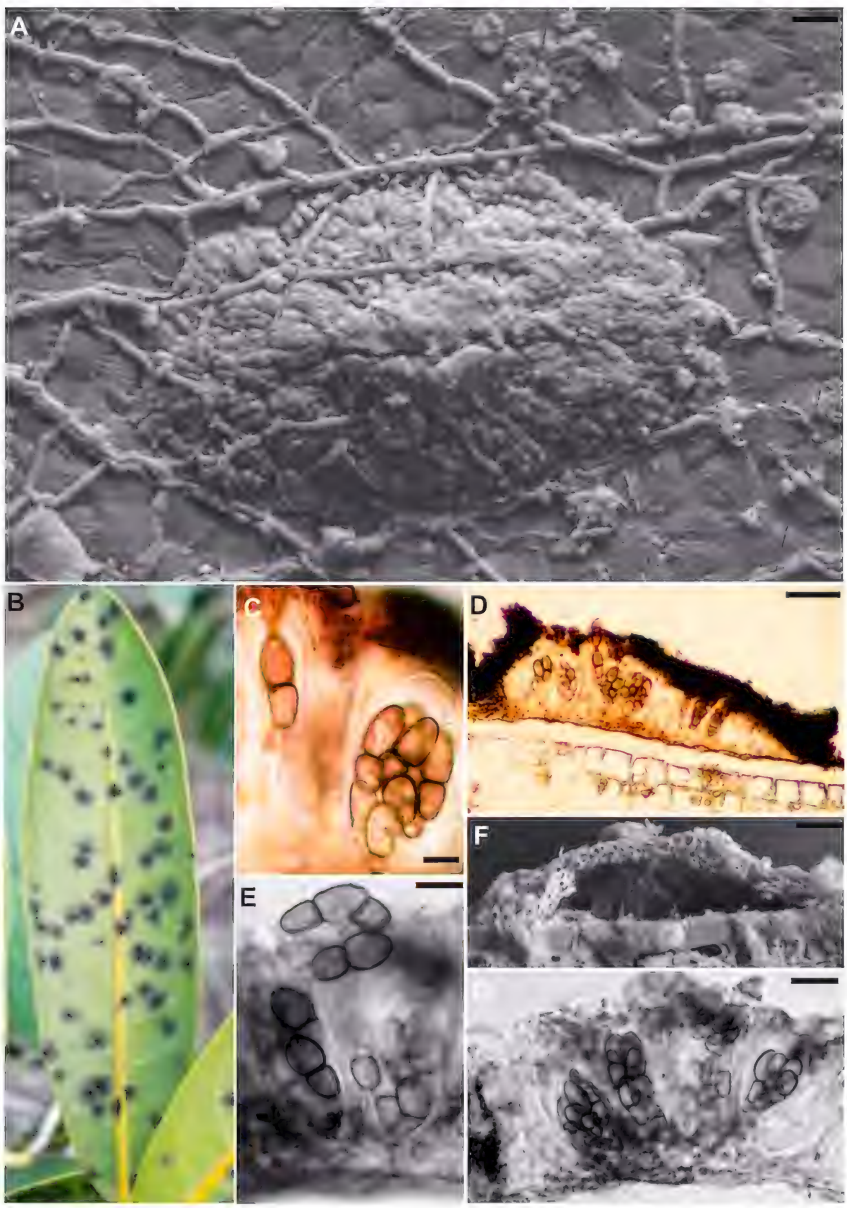

Plate 1. *Lembosia dianesei* (VIC 42934) on *Peritassa campestris*. A: hysteriothecia and mycelium (SEM). B: epiphyllous colonies. C, G: asci and ascospores. D, E: section through ascoma. F: uniseptate ascospores, constricted at the septum. Scale bars: A, F = 10 μm; C–E, G = 40 μm.

Etymology: In honor of the Brazilian mycologist, José Carmine Dianese.

Colonies epiphyllous, irregular or circular, single or confluent, black color, hyphae straight flexuous, branched at 45° angle, brown, septate, hyphal cells cylindrical 3.4–5.3 µm diam., smooth. Hyphopodia (appressoria) numerous, lateral, alternated and unilaterally distributed, nodule-shaped, unicellular, 4.5–7.1 × 4.7–6.6 µm, brown, penetration peg central on the appressorial cell. No haustoria were observed. Ascomata superficial, hysteriothecia, single to confluent, fringed at margins, linear or Y–X shaped, 150–335 × 130–160 µm, with a height of 29–70.5 µm, opening by a longitudinal dehiscence, dark brown; upper wall of textura prismatica, cells radiating, rectangular, 6.5–13.2 µm thick, brown; basal wall 3.5–16.3 µm thick. ASCI bitunicate, disposed as an upright palisade layer, ellipsoid to subglobose, 8-spored, hyaline to brown, 28.7–36.3 × 14.4–19.2 µm. Ascospores oblong to ellipsoid, ends rounded, straight, 1-septate, slightly constricted at the supra-median septum, hyaline when young and brown at maturity, smooth, conglobed, 19.7–28.7 × 6.8–10 µm; apical cell smaller, basal cell larger and thinner. Asexual morph not seen.

Additional specimen examined: **BRAZIL**, **Minas Gerais**, **Botumirim,** on living leaves of *Peritassa campestris*, 30 July 2015, M.P. Melo (VIC 42935).

Comments

Three *Lembosia* species have been previously reported in association with living leaves of celastraceous hosts: *L. salaciae* Hosag. & Archana, associated with *Salacia* sp. from India (Hosagoudar & Archana 2009); *L. albersii* Henn., associated with *Elaeodendron* sp. from Tanzania (Hennings 1902); and *L. lophopetali* (Rehm) Hansf., associated with *Lophopetalum toxicum* from the Philippines (Hansford 1949). Three additional records of *Lembosia* spp. have been reported from Brazil associated with living leaves of undetermined (or unstated) celastraceous hosts (Farr & Rossman 2015).

The three previously described species are easily distinguished from *Lembosia dianesei* (Table 1): *L. albersii* differs by its larger asci and its median ascospore septum (Hennings 1902); *L. lophopetali* differs by its longer two-celled hyphopodia, its larger asci, and its wider ascospores (Hansford 1949); and *L. salaciae* differs by its longer two-celled hyphopodia and its larger ascomata, asci, and ascospores (Hosagoudar & Archana 2009). Another peculiar characteristic of *Lembosia dianesei* is its strong hyphal adherence to the foliar surface.

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The authors thank Jaime Pereira de Melo, a technician at the Empresa de Extensão Rural do Estado de Minas Gerais (EMATER-MG), for his support in collecting

Table 1. Comparisons of hosts and morphology in *Lembosia* species associated with *Celastraceae*

Species	Host	Hyphopodia (µm)	Ascomata (µm)	Asci (µm)	Ascospores (µm)
L. dianesei	*Peritassa campestris*	4.5–7.1	205–335 × 130–160	28.7–36.3 × 14.4–19.2	19.7–28.7 × 6.8–10
L. salaciae	*Salacia* sp.	9–18	500–560 × 250–300	32–42 × 26–35	28–35 × 12–18
L. albersii	*Elaeodendron* sp.	—	—	45–90 × 20–30	20–30 × 10–12
L. lophopetali	*Lophopetalum toxicum*	—	—	40–50 × 20–25	25–30 × 11–14

specimens, Capes and CNPq for the scholarship given to J.L. Bezerra, and the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support. They are also grateful to Jadergudson Pereira (Universidade Estadual de Santa Cruz, Brazil) and Olinto Liparini Pereira (Universidade Federal de Viçosa, Brazil) for helpful comments and presubmission review.

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Regional annotated mycobiotas new to the Mycotaxon website

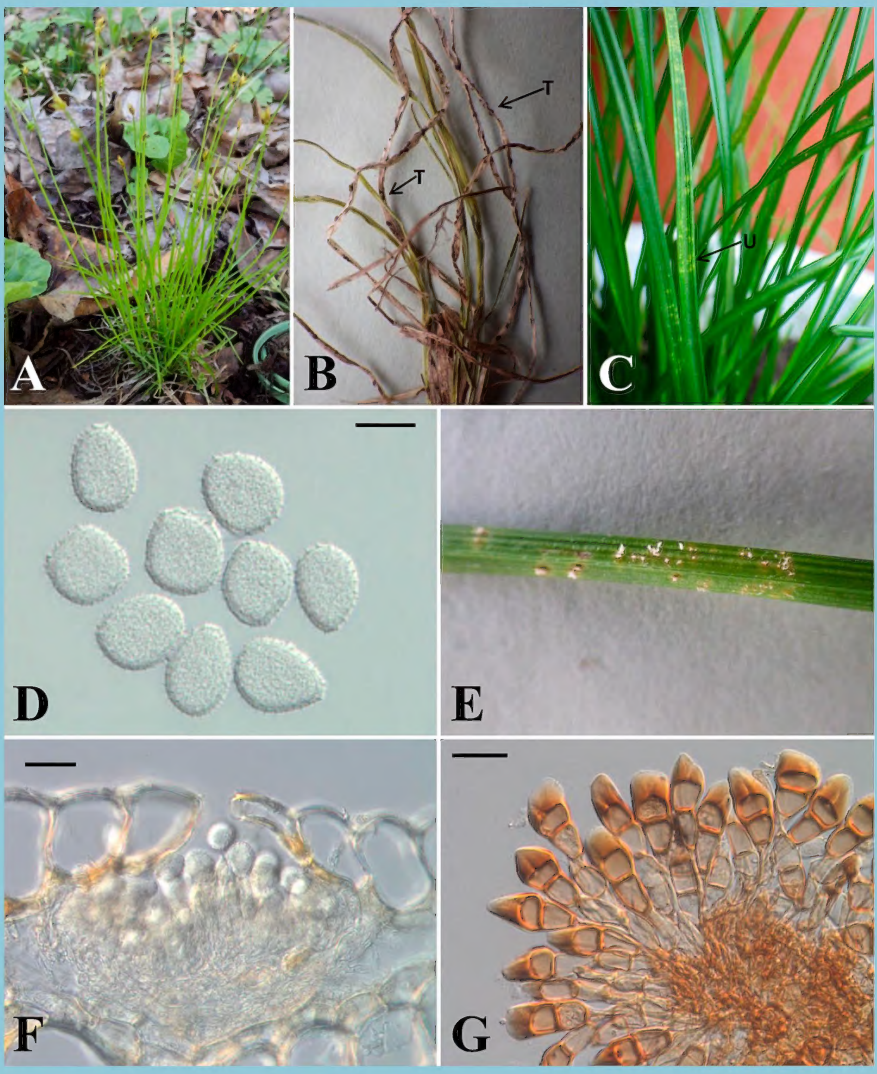
Abstract—Mycotaxon is pleased to announce another new species distribution paper to our 'web-list' page covering clavarioid fungi in Brazil (by Meiras-Ottoni, Araujo-Neta & Gibertoni). This brings to 124 the number of free access mycobiotas now available on the Mycotaxon website: http://www.mycotaxon.com/resources/weblists.html

South America

Brazil

Angelina de Meiras-Ottoni, Lidia Silva Araujo-Neta & Tatiana Baptista Gibertoni. A checklist of clavarioid fungi (*Agaricomycetes*) recorded in Brazil. 22 p.

Abstract—Based on an intensive search of literature about clavarioid fungi (*Agaricomycetes: Basidiomycota*) in Brazil and revision of material deposited in Herbaria PACA and URM, a list of 195 taxa was compiled. These are distributed into six orders (*Agaricales, Cantharellales, Gomphales, Hymenochaetales, Polyporales* and *Russulales*) and 12 families (*Aphelariaceae, Auriscalpiaceae, Clavariaceae, Clavulinaceae, Gomphaceae, Hymenochaetaceae, Lachnocladiaceae, Lentariaceae, Lepidostromataceae, Physalacriaceae, Pterulaceae,* and *Typhulaceae*). Among the 22 Brazilian states with occurrence of clavarioid fungi, Rio Grande do Sul, Paraná and Amazonas have the higher number of species, but most of them are represented by a single record, which reinforces the need of more inventories and taxonomic studies about the group.



Puccinia adenocauli comb. nov.
(Ji & al.—Fig. 2, p. 146)